Chapter 1 Introduction

Research problem

Benzene, a volatile organic compound, is extracted from petroleum industries and widely used as an additive, an intermediate, and/or a solvent in many manufacturing industries. Nowadays, the emission of benzene from many sources can cause ambient air pollution problems, even though several organizations and countries have standard guidelines on ambient benzene concentrations (Pollution Control Department 2007; World Health Organization 2000). Benzene concentration in the atmosphere was found to be higher than the local standard guidelines in many places around the world. In addition, 9 cohort and 13 case-control studies confirm that benzene can clearly induce acute myelogenous leukemia and other cancers (Schnatter et al. 2005). From many researches, benzene is classified in the 1A group, which is composed of high potential carcinogens in the human body, by IARC (Guieysse et al. 2008). Allergies, asthma, dizziness, tremors, restlessness, eye irritation, and nervous-system disorders were also known to be caused by benzene (Wolverton 1996). Benzene can also be accumulated and stablized in the environment.

There are several technologies that have been applied to removal benzene, but most of these technologies request high cost and skill to control. In addition, these technologies can be used only on industries. For indoor benzene accumulation in home or office, benzene removal by plants was interested and studied, and strong evidence showed that some species of plants could uptake benzene well (Liu et al. 2007; Orwell et al. 2004; Wolverton et al. 1989). However these experiments had been done in European and US, which have difference environmental condition with Thailand. Affecting factor for examples temperature, pressure, etc. can effect to benzene uptake by plant. The study on benzene phytoremediation including screening high benzene removal efficiency plants in Thailand, benzene uptake pathway in plants, sustainability of benzene phytoremediation, and affecting factor is an important issue that should be investigated.

Moreover, Ugrekhelidze et al. (1996) reported that plants could uptake gaseous benzene through the stomata and wax on the surface of the leaf. The benzene accumulation in cuticular wax of plant leaves was found in many researches (Collins et al. 2000; Environmental Agency 2009; Gorna-Binkul et al. 1996; Kylin et al. 1994; Poborski 1988; Riederer 1990; Slaski et al. 2000; Tsiros et al. 1999). Benzene that contains 2.13 of log

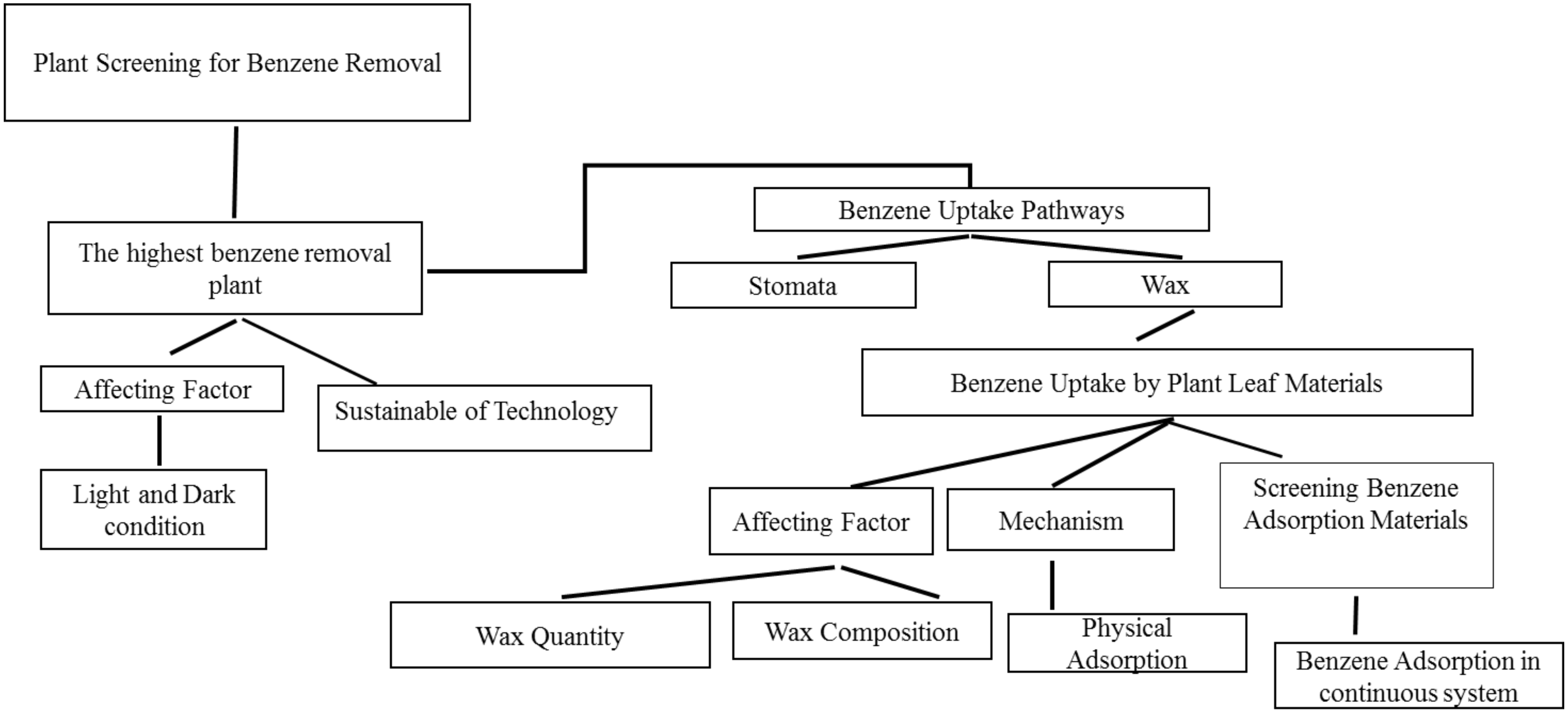
Kow, a logarithm value of Octanol/water partition coefficient, can transport easily into the plant (Kamath et al. 2004). Nowadays, activated carbon was widely applied to treat benzene however high cost for control and secondary waste disposal was a problem. The use of plant leaf material for benzene adsorption was interested as a low cost adsorbent. However, a few studies reported on the application of plant leaf material for benzene adsorption. In this present study, several plant leaf materials were screened for benzene adsorption in a close system. The composition of wax might be more important than quantity of wax in benzene adsorption (Topp et al. 1986), so not only new adsorbent from plant leaf material was improved but the effect of wax quantity and composition of wax of each plant leaf material was analyzed as criteria for the selection of plant materials for benzene adsorption.

Objectives

1. To screen ornamental plant for benzene removal and investigate sustainability of benzene phytoremediation and benzene uptake pathway on plant.
2. To screen plant leaf materials for benzene adsorption and analyse the relation of cuticle quantity and composition on benzene adsorption.

Scope of The experiment

1. Eight ornamental plants were screened to remove gaseous benzene in close system.
2. Benzene removal under light and dark conditions by *Dracaena sanderianaI*, which is highest benzene removal plant, was studied.
3. Sustainability of benzene removal by *Dracaena sanderiana* was investigated.
4. Wax quantity, stomata pattern, and PhotosystemII activity were analysed to study factor affecting on benzene removal efficiency.
5. Leaf materials from 21 species of plant was prepared and screened to adsorb benzene.
6. Wax quantity and composition were analysed and used to calculate the relationship between benzene removal efficiency and wax quantity and wax composition.
7. Effective 6 plant leaf materials were applied to use in continuous system.
8. Benzene adsorption mechanism of plant leaf materials was studied.


Plant Screening for Benzene Removal
The highest benzene removal plant
Benzene Uptake Pathways
Stomata
Wax
Affecting Factor
Sustainable of Technology
Light and Dark condition
Benzene Uptake by Plant Leaf Materials
Affecting Factor
Mechanism
Screening Benzene Adsorption Materials
Wax Quantity
Wax Composition
Physical Adsorption
Benzene Adsorption in continuous system

Chapter 2 Literature review

Benzene

Physical and chemical properties of benzene

Benzene, which has been normally classified as one of volatile organic compounds (VOCs) because of high vapours pressure, small molecule, and evaporation at room temperature, has ring conformation with resonance structure, so this compound could be stable in the environment. The compound contains 6 C and 6 H atoms (C_6H_6) that had been shown in Figure 2.1.

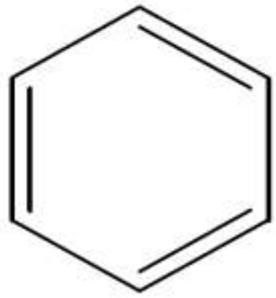


Figure 2.1 Benzene molecules.

Benzene is a hydrophobic compound that could be solubilized easily in oils, hexane, ethanol, and chloroform etc. This compound had been known commonly as explosive vapours and flammable liquid. Physical and chemical properties are presented in Table 2.1 (Sciencelab, 2001). The solution of benzene appears in colourless liquid with sweet odour. The compound could evaporate 100% at room temperature because of high vapours density. Benzene could be solubilized in n-octanol more than in water around 100 times as showing in log K_{ow} that equal to 2.13.

Table .21 Physical and chemical properties of benzene (Sciencelab, 2001).

Physical and chemical properties	
Physical state and appearance	Clear liquid
Color	Colorless
Odour	Sweet, solvent-like
Odor threshold	1.5-5 ppm
Vapours density at 0°C	2.8
Boiling point	80°C
Melting point	5.5°C
Solubility	0.1-0.3% in water
Specific gravity	0.88 at 15 °C
log K_{ow} (octanol/water coefficient)	2.13
Percent volatile	100
Flammability classification	Flammable liquid

In general, benzene could be extracted from petroleum industries (U. S. Environment Agency, 2009). Oil distillation could classify petroleum product such as fuel oil, wax, lubricants, diesel oil, kerosene, gasoline, and petroleum gases. Benzene that contains 6 carbon atoms could be extracted from the group of gasoline, which identify normally to the group of 5-12 carbon atoms (Fig 2.2).

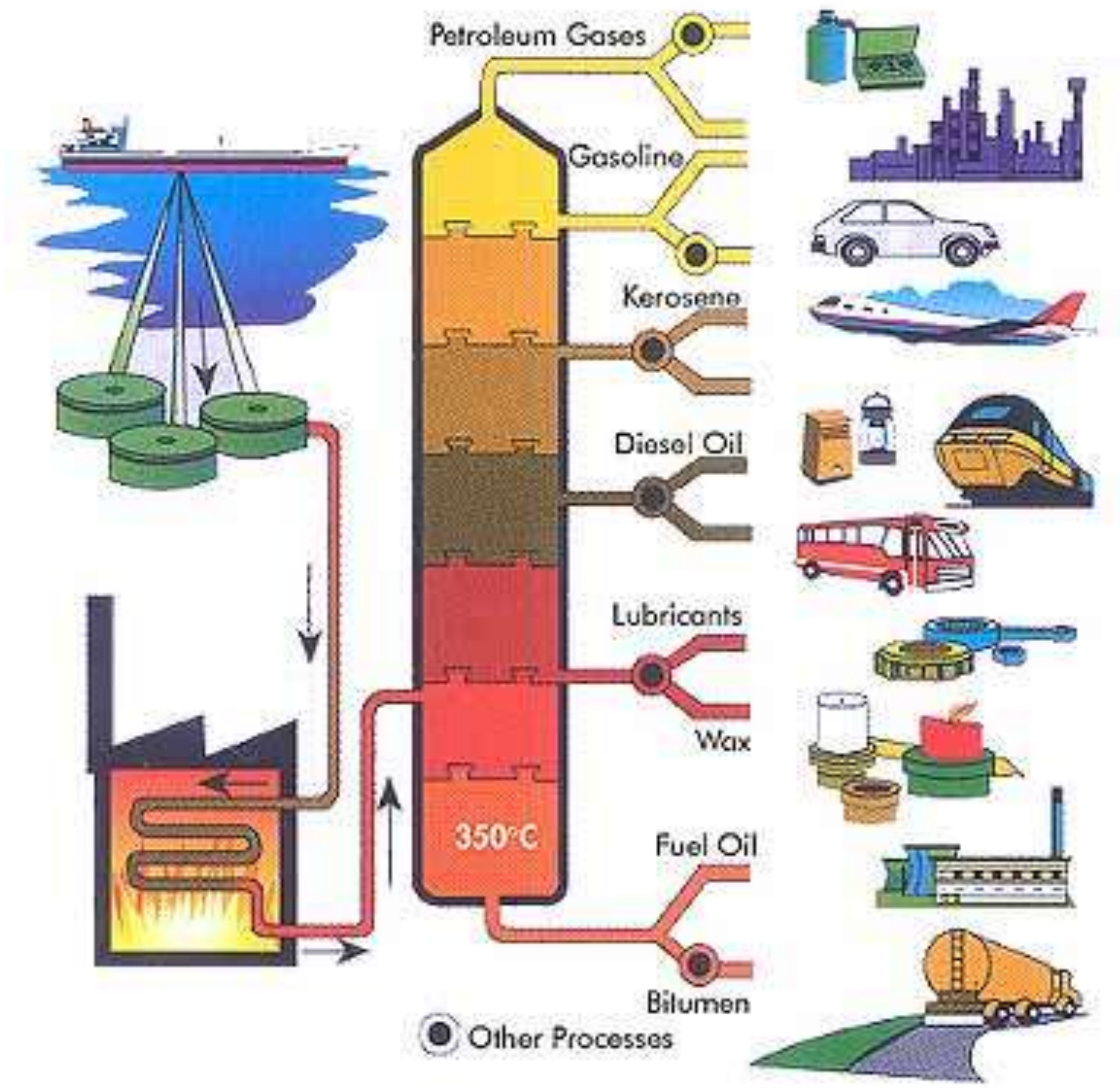


Figure 2.2 Petroleum distillations (Sapref, ND).

Benzene has been widely used as additives, intermediate and/or solvent in several industries for example coating compounds, chemical synthesis, solvent in laboratory, etc. (Atkinson, 2007), so benzene might contaminate in the environment and food chain and create disease and/or symptoms in human.

Source of benzene

There are several source of benzene, for example combustion, petroleum industries, and vehicle. From many survey studies, the results suggested that main source of benzene depend on the structure and composition of the city. For example, previous observation in Thailand showed that vehicle was the most emission source of benzene about 78% of total benzene emission. The result is shown in Table 2.2 (Pollution control department, 2007).

Table 2.2 Percentage of benzene emission sources in Thailand (Pollution control department, 2007).

Sources	Emission (Ton/Year)	%
Vehicle	1588	77.5
(Gas/uncontrol)	1343	65.6
(Diesel)	181	8.8
(Gas/control)	64	3.1
Industries combustion sources	122	6
Gasoline storage and transportation	323	15.8
Other sources	15	0.7

Although most of benzene had been emitted from vehicle in Thailand, all sources of benzene emission could cause for ambient and indoor air quality problem (Wolverton, 1996). Moreover, sources of benzene emission in Canada, the survey results suggested that more of benzene had been emitted from transportation (Fig 2.3).

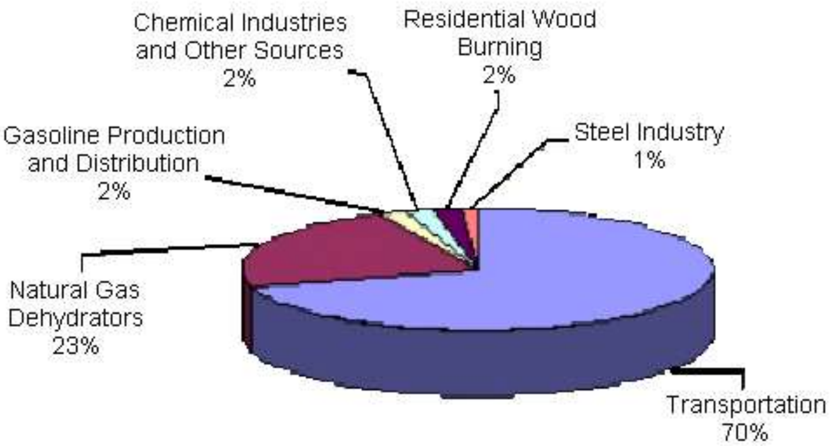


Figure 2.3 Percentage of benzene emission sources in Canada (Canadian Environmental Protection Act, 1999).

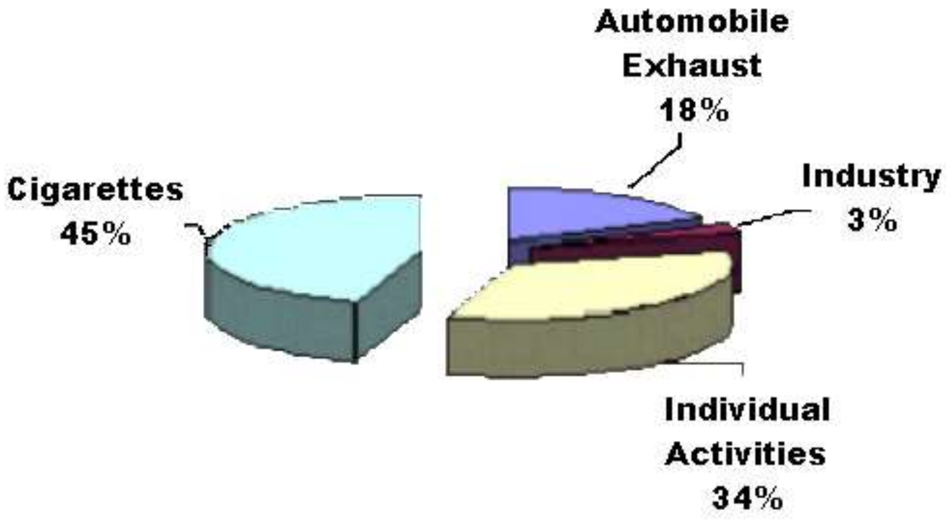


Figure 2.4 Percentage of human benzene exposure sources in Canada (Canadian Environmental Protection Act, 1999).

However, the most important source of benzene exposure in human is not from vehicles, but cigarettes had been reported as the highest percentage of source of benzene uptake by human, which is 45% of total benzene exposure. Automobile exhaust had been reported as a source of benzene exposure in human only 18% (Fig 2.4). Not only direct emission source but also indirect emissions source such as consumer product in home or office could be also found (Table 2.3). Poor ventilation is commonly known as properties of indoor air. Accumulation of benzene in poor ventilation space had been considered to be important problem (U. S. Environment Protection Agency, 2007).

Table 2.3 VOCs emission from each consumer product (U. S. Environment Protection Agency, 2007).

	Emission					
Sources	**Formaldehyde**	**Xylene/toluene**	**Benzene**	**Ammonia**	**Alcohols**	**Acetone**
Adhesive	•	•	•			
Bio effluents		•		•	•	•
Carpeting					•	
Caulking compounds	•	•	•		•	
Ceiling tiles	•	•	•		•	
Cleaning product				•		
Cosmetics					•	•
Draperies	•					
Electro photographic printers		•	•	•		
Fabrics	•					
Facial tissue	•					
Floor covering	•	•	•		•	
Grocery bags	•					
Nail polish remover					•	
Office correction fluid					•	
Paints	•	•	•		•	
Paper towels	•					
Particleboard	•	•	•		•	
Photocopies		•	•	•		
Pre-printed paper forms						•
Stains and carnishes	•	•	•		•	
Upholstery	•					
Wall covering		•	•		•	

Benzene toxicology

The use of benzene in manufacturing and vehicle could create benzene contamination in ambient air, emission air, indoor air, and consumer product. Actually, there are 4 benzene uptake pathways in human such as skin absorption, skin adsorption, gastrointestinal tract, and respiratory system (Fig 2.5). Gastrointestinal tract and respiratory system had been known as the main benzene pathways (Sciencelab, 2001). In benzene uptake by gastrointestinal tract, because benzene molecule is normally stable in environment and shows hydrophobic properties, the molecule could be easily accumulated in non-polar part of living cell. The eating of benzene contaminated food, the molecule could be diffused rapidly in to the human body. For the respiratory system, gaseous benzene could be breathed into human lung, and the molecule could be passively absorbed (Rappaport *et al.*, 2012). The emission of benzene from the consumer product appear and accumulate in indoor space, and long term benzene exposure in poor ventilation space, many diseases could be induced that cause people who mostly spend in indoor. The liver, kidneys and central nervous system damage could be generated by benzene (U. S. Environment Agency, 2007). From several studies had confirmed that benzene could be also human carcinogenic molecule, so many organization had classified mostly benzene in dangerous compound group. The International Agency for Research on Cancer (IARC) had classified benzene in group1 that prefer to human carcinogenic. Benzene is grouped in group A by USNTP of USEPA as well that main the agent could be carcinogenic to humans with enough epidemiological evidences. ACGIH and JSOH also classified benzene in group 1A and 1, respectively those are human carcinogenic group (Pollution control department, 2007). For example, one of benzene induced cancer, which is white blood cell cancer in the type of Acute Myelogenous Leukemia (AML), could be induced surely by long-term benzene exposure (Schnatter *et al.*, 2005). Not only chronic diseases but also acute diseases could be induced from high concentration of benzene exposure. Nose discomfort, headache, allergic skin reaction, emesis, dizziness, etc. are the symptoms that happen by short term high benzene concentration exposure (U. S. Environment Agency, 2007). For the suggestion, mouse 50% lethal dose (LD_{50}) and Immediately Dangerous to Life or Health (IDLH) are about 930 mg kg^{-1} and 500 ppm, respectively (Sciencelab, 2001). Not only human toxicology but also environment toxicology was proposed. Benzene is strong aromatic ring conformation, so it is very stable compound. The environmental accumulation of benzene is also happened, and it can affect to animal, fish and some microorganisms (Sciencelab, 2001).

Standard of benzene

Nowadays, several organizations and countries have realized benzene contamination problem in environment, so benzene concentrations have been tried to regulate by many standards. In difference areas such as workspace and ambient, difference values of benzene concentration has been guided however emission standard of benzene from industries are not found. The guideline of benzene in ambient air, workspace, and also water are shown in Tables 2.4 -2.6, respectively.

Table 2.4 International available of ambient benzene (Pollution Control Department, 2007).

Organization		Benzene (μg m^{-3})
WHO Guideline for Air Quality (2000)		5~20
New Zealand		10 (annual)
Canada		-
Japan		3 (annual)
California		-
Rhode Island Air Toxic Guideline	1 hour	200
	24 hour	10
	Annual	0.1
Arizona	1 hour	630
	24 hour	51
	Annual	0.14

Table 2.5 Internationally available of work space benzene concentration (Pollution Control Department, 2007).

Organization	Exposure time	Benzene (ppm)
OSHA (Work space)	8 h/day	1
	15 min	5

Table 2.6 Internationally available of water contamination with benzene (Pollution Control Department, 2007).

Organization	mg L^{-1} (Water)
USEPA	0.005

Thailand situation

Because benzene has been applied to be a additive and/or a solvent in several industries, Thailand manufacturing also use this compound. From demand-supply of benzene data in 2001-2005, the tendency of benzene production had increased from 502,000 tons in 2002 to 742,000 tons in 2005 (Pollution Control Department, 2007). The quantity of imported benzene is ranked as top 10 in CMR (C: Carcinogen, M: Mutagenic, R: Reprotoxic) group in 2002 and 2005. Benzene contamination from several sources can create environmental problem, so pollution control department, Thailand regulate benzene concentration in ambient air when exposure to 24 h accumulation is about 7.8 µg m^{-3} and 1.7 µg m^{-3} for 1 year exposure (Pollution Control Department, 2007). Demand-supply of benzene in 2001-2005 is shown in Figure 2.6.

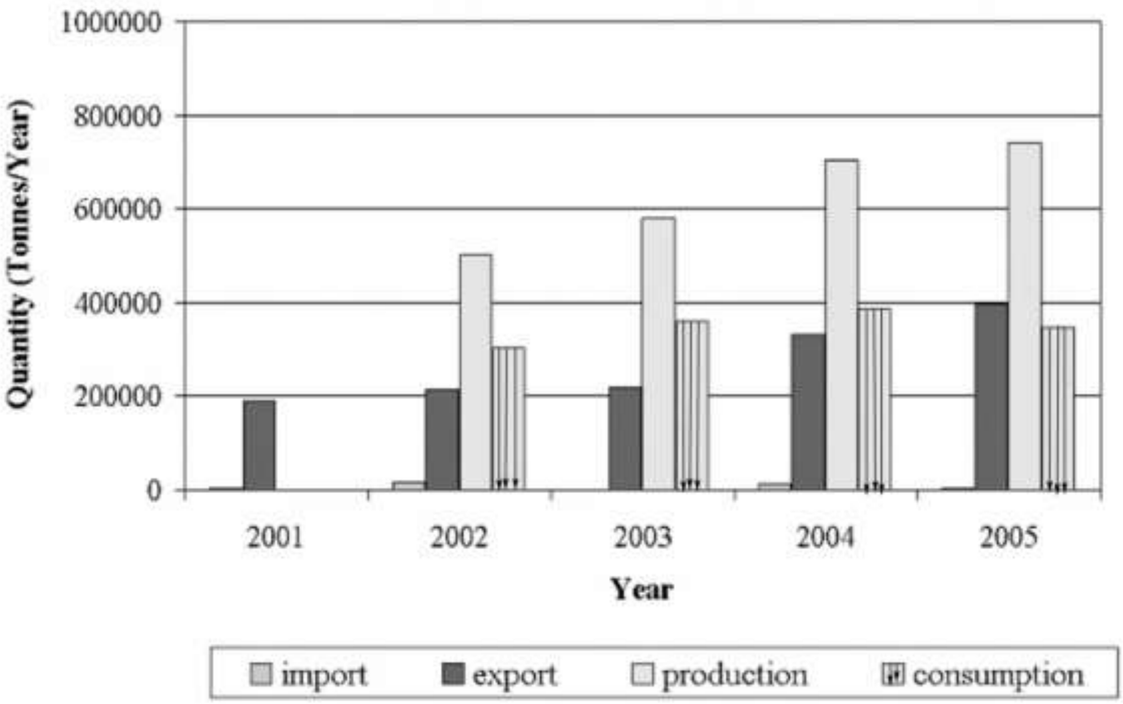


Figure 2.5 Demand-supply of benzene in 2001-2005 (Pollution Control Department, 2007).

The accumulation of benzene had been considered as important problem because although pollution control department of Thailand has benzene standard guide line, higher benzene concentration than standard had been found in many locations around Thailand especially in Bangkok and industrial cities (Fig 2.7).

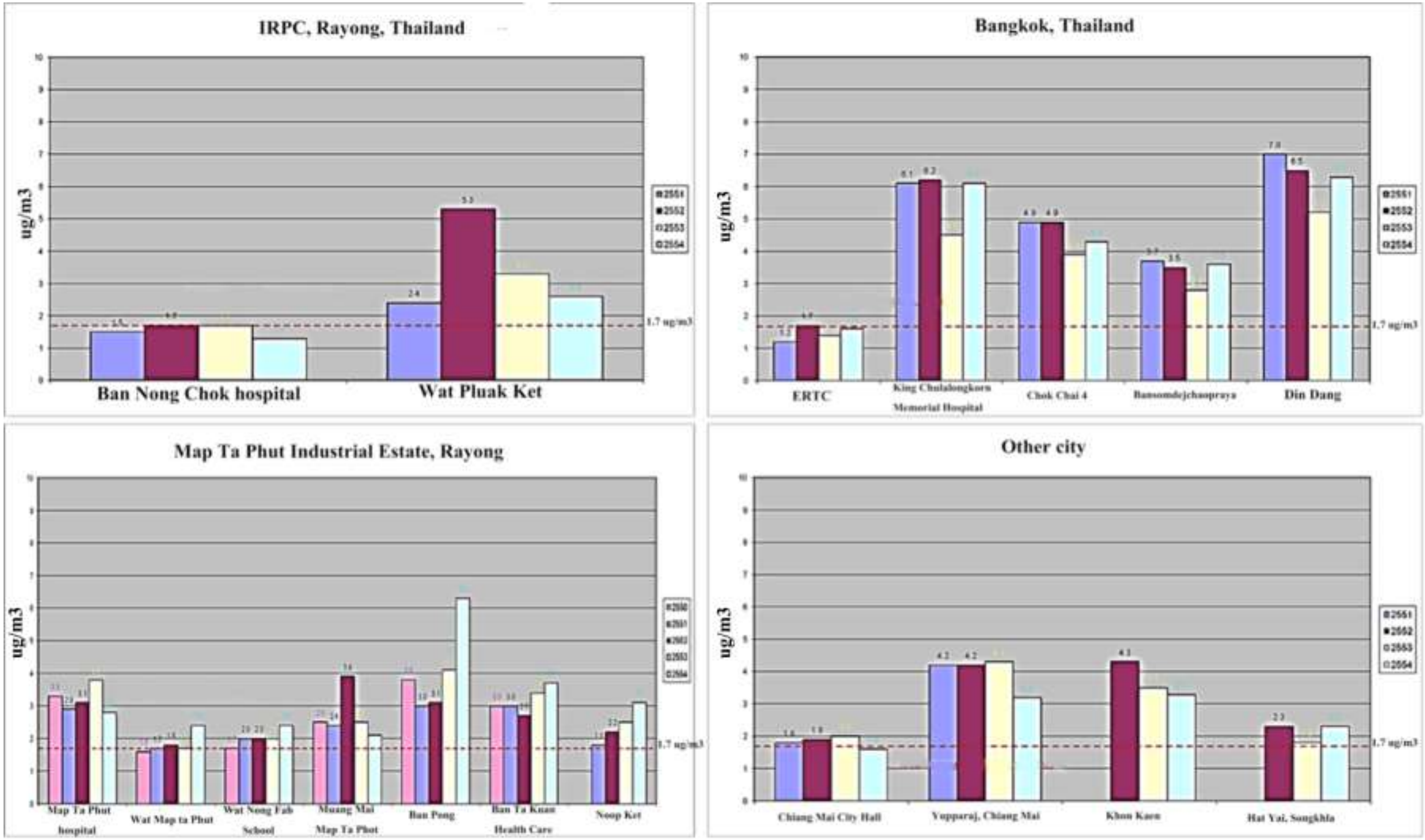


Figure 2.6 Benzene concentration in ambient air around Thailand, 2008-2011 (Pollution Control Department, 2007).

Adsorption and absorption

Adsorption and absorption process have been generally applied to treat air pollution in several industries. These processes could reduce accumulated energy of pollutant molecule or create bond to pause molecule mobilization. The target molecule could be adhered on the surface of absorbent or adsorbent. For the adsorption, pollutant could be fixed onto a solid matrix typically a surface or a porous material (Michel, 2008). Accumulated energy of pollutant would be decreased when the molecule had been fixed onto the adsorbent. Moreover, the thermodynamic equilibrium is di-variant. In case of absorption, physical or chemical phenomenon or a process in atoms, molecules, or ions enter some bulk phase that might be liquid, solid, and gas. This process, mobilization of pollutant might be slowed down and adhere in to bulk phase of absorbent, which also include the penetration to intra-matrix of absorbent. Also chemical reaction, absorption process might create chemical bond to combined pollutant molecule (McMurry, 2003). The difference between these processes is that in adsorption, pollutant would be adhere only on the surface of matrix of adsorbent, but absorption, the process include also penetration of pollutant to intra-matrix of absorbent (Fig 2.8).

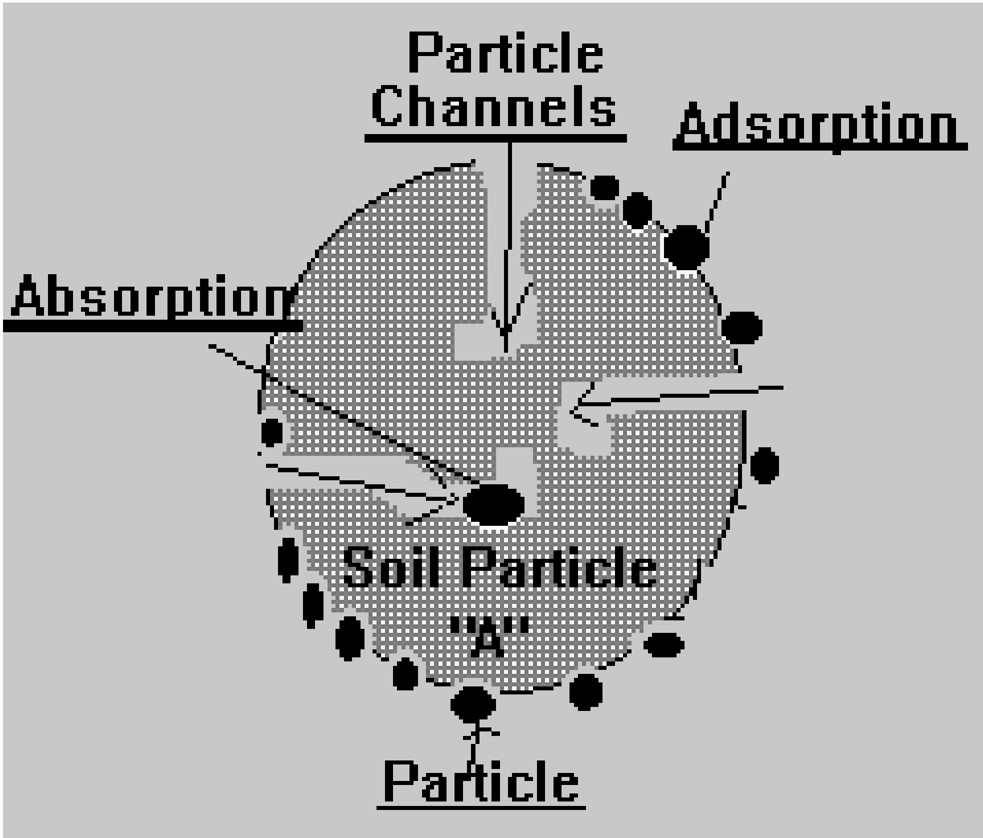


Figure 2.7 Adsorption and absorption process (McMurry, 2003).

Activated carbon has been sometimes called activated charcoal or activated coal. The structure is a form of carbon that has been processed to make extreme porous and create a very large surface area available for adsorption or chemical reactions. Chemical activation and steam activation techniques are normally used by operations of commercial production. The activation of peat and wood based raw materials; Chemical activation is generally used at temperature of 500 – 800 ^{o}C with mixed phosphoric acid (H_3PO_4) or zinc chloride ($ZnCl_2$) into a paste. Chemical activation process could generate macro-porous activated carbons for the large molecules absorption. In steam activation, Coal and coconut shell are used as raw material. Temperatures of 800 – 1100 ^{o}C in the presence of steam have been normally applied to activate the absorbent. Micro-porous and meso-porous are created in this process. Type of raw material available, desired physical form of the activated carbon, characteristics required for the intended application are the factor for activation technique selection. Activated carbon could be classified following a highly internal pore structure and surface area. The different characteristics depend on the raw material and activation techniques (Caron Link, 2011). The International Union of Pure and Applied Chemistry (IUPAC) define the pore size distribution as:

The macro-pores could be used as the entrance to the activated carbon and large molecule absorption. The meso-pores are important structure for transportation, and finally, the micro-pores could be used to absorb small molecular weight compound. In air pollution treatment, high pollutant removal efficiency and fast absorption was found in micro-pores activated carbon especially benzene, so activated carbon with high micro has been widely used to treat contaminated pollutants in industries. However the adsorption efficiency of activated charcoal depend on concentration of pollution, temperature of vapours stream, relative humidity of vapours stream, flow rates and operating frequency, process operating pressure, pressure drop in system. The activated carbon capacity for chemical fumes, gas, and odors had been classified by the numbers 1 – 4. The description of numbers 1 – 4 was shown in Table 2.7. Some of the contaminated pollutants had been listed and classified following the number of activated carbon capacity in Table 2.8.

Table 2.7 Capacity index numbers and descriptions (Caron Link, 2011).

Index No.	Capacity	Notes
4	High, one pound of carbon can adsorb approx., 20% to 50% of its own weight	Includes most odor causing substances
3	Satisfactory, one pound of carbon can adsorb approx., 10% to 20% of its own weight	Capacity is not high as 4
2	Not highly adsorbed	Might be taken up sufficiently under particular conditions of operation
1	Low	Activated carbon cannot be used satisfactorily to remove chemical gas under ordinary condition

Table 2.8 Chemical absorption index of activated charcoal / activated carbon (Caron Link, 2011).

Chemical	Index number	Chemical	Index number	Chemical	Index number	Chemical	Index number	Chemical	Index number
Acetic acid	4	Carbon disulfide	4	Ethyl chloride	3	Methyl acrylic	3	Nonane	4
Acetic anhydride	4	Carbon dioxide	1	Ethyl ether	3	Methyl alcohol	3	Octalene	4
Acetone	3	Carbon monoxide	1	Ethyl formate	4	Methyl bromide	4	Octane	4
Acetylene	1	Carbon tetrachloride	4	Ethyl mercaptan	1	Methyl butyl ketone	4	Ozone	4
Acrolein	3	Chorine	3	Ethyl silicate	1	Methyl chloride	4	Pentane	3
Acrylic acid	4	Chlorobenzene	4	Ethylene	4	Methyl chloroform	3	Pentanone	4
Acrylonitrile	4	Chlorobutadiene	4	Ethyl chlorhydrin	4	Methyl ether	4	Pentylene	3
Alcoholic beverage	4	Chloroform	4	Ethyl dichloride	3	Methyl ether ketone	3	Pentyne	3
Amines	2	Chloronitropropane	4	Ethyl oxide	4	Methyl formate	4	Perchloroethylene	4
Ammonia	2	Chloropicrin	4	Flurotrichloromethane	3	Methyl iso butyl ketone	4	Phenol	4
Amyl acetate	4	Crotonaldehyde	4	Formaldehyde	2	Methyl mercaptan	4	Propane	2
Amyl alcohol	4	Dichloroethylene	4	Formic acid	3	Methylcyclohexane	4	Propionaldehyde	3
Amyl ether	4	Dichloroethyl ether	4	Heptane	4	Methylcyclohexanol	4	Propionic acid	4
Aniline	4	Dichloropropane	4	Heptylene	4	Methylcyclohexanone	4	Propyl chloride	4
Benzene	4	Dichloromonofluomethane	4	Hexane	3	Methyl oxide	1	Propyl ether	4
Borane	3	Diethylamine	4	Hexylene	3	Methylene chloride	4	Propyl mercaptan	2
Bromine	4	Doethyl ketone	4	Hexyne	3	Methylmethacrylate	4	Propyne	2
Butadiene	3	Dimethylaniline	4	Hydrogen	1	Monochlorobenzene	4	Radiation product	2
Butane	2	dimethylsulfate	4	Hydrogen bromide	2	Monoflurotrichloromrthane	4	Sulfur dioxide	3
Butanone	4	Dioxane	4	Hydrogen chloride	2	Naptha	4	Sulfur trioxide	4
Butyl acetate	4	Dipropyl ketone	3	Hydrogen cyanide	3	Napthalene	4	Tetrachlorethylene	4
Butyl chloride	4	Ethane	4	Hydrogen sulfide	4	Nicotine	4	Toluene	4
Butyl ether	4	Ether	4	Iodine	4	Nitric acid	3	Toluidine	4
Butylene	2	Ethyl acetate	4	Iodioform	4	Nitro benzene	4	Trichloroethylene	4
Butyne	2	Ethyl acrylic	3	Isophorone	3	Nitroethane	4	Trichloroethane	4
Butyraldehyde	3	Ethyl alcohol	4	Isoprene	4	Nitrogen oxide	2	Uric acid	4
Butyric acid	4	Ethylamine	4	Isopropyl alcohol	1	Nitroglycerine	4	Valeric acid	4

Phytoremediation

The use of plants to clean-up the environment, which is called phytoremediation, is one of an effective treatment. In general, phytoremediation had been used for metal cleaning in underground water, surface water, and soil. In addition, VOCs removals by plants had been studied. Pollution degradation, stabilization, or evaporation in soil, sediment, ground water, and also atmosphere could be successes by this method. Low cost, easy to maintain, and no secondary pollutants are the advantages of this method. However; this method requires long time, large lands, and the efficiency relate with spices of the plants. One of important limitation of this method is that plant could not survive under high concentration of toxic compound. Phytoremediation could be classified in many types such as phytoextraction, phytodegradation, phytostabilization, rhizofiltration, phytovolatilization, rhizodegradation. The detail of each method is as follows.

Phytoextraction

Phytoextraction or phytoaccumulation, which is a type of phytoremediation, usually could be used to control metal contaminating land. This method, plants could uptake toxic metal by roots and accumulate the toxic molecules in a part of plant which upon to plant species and metal type. The ion of metal that could solubility easily in water would be transport rapidly to the shoots and leaves of plant. However most of experiment showed that plants accumulated commonly metal in the roots. In this type of phytoremediation, only direct accumulation of metal has been considered.

Phytostabilization

Phytostabilization is a method for inhibition of pollutant distribution in soil and water. In general, plants uptake toxic substances for example lead, cadmium, arsenic, etc. by roots. Toxic substance could be transformed and conjugated to biomolecule, and this process makes normally high stability form of toxic compounds. High stability compounds might accumulate in plant cell organelle or precipitate in the environment, so this method could control distribution of contaminants in the environment. Moreover, phytostabilization could be also processed in both inorganic and organic compounds.

Rhizofiltration

Most of soil and water pollutant could be uptake and reduced rate of pollution distribution by plant roots, which is called rhizofiltration. This process is not only filtrate but also include contaminants absorption. Pollution distribution rate could be reduced by this type of phytoremediation, and some pollution could be uptake and accumulated by plant roots. However filtration rate relates with species of plant and toxic substances.

Phytovolatilization

Some of toxic molecules could be transported from soil and water to atmosphere by plants, which might be called phytovolatilization. The compounds would be uptaken by plant roots and transported to plant shoots and leaves. Conformation of some toxic compounds would be changed to easy evaporated form and emitted to atmosphere through stomata of plant leaves.

Rhizodegradation

Many names could be presented the similar meaning with rhizodegradation such as phytostimulation, rhizosphere biodegradation or enhanced rhizosphere rhizodegradation. This process, the relation of plant and microorganism such as yeast, fungi, and bacteria has been presented. Some essential microorganisms that grow around plant roots could uptake and degrade toxic substances for growing. Some organic compounds could be used as carbon and energy sources for microorganism. Not only pollutants degradation by microorganism but also plant growth promoting could be considered as a benefit of essential microorganism.

Phytodegradation

Phytodegradation, which could be called phytotransformation, is a method for toxic substances degradation by plants. In this process, plants would generate specific catalytic enzymes that suitable for the toxic compound degradation. This type of phytoremediation would happen mostly in organic compounds. Some small molecules, plant could degrade completely to carbon dioxide or essential organic acid in tri-carboxylic acid cycle (TCA cycle). However some complex molecules that contain metal, large molecule or other, plant could only stabilize them after that the stable compound might excrete back to environment or accumulate in plant cells.

Green liver concept for organic compounds

Principle of chemical compound degradation and/or detoxification in plant had been shown in the model, which could be called "green liver concept". This concept show normally principle of mobilization of compound in plant cells that toxic compounds could be generally separated as heavy metals and organic compounds. In this case, only organic compounds had been presented that normally, organic compounds might be degraded completely to carbon dioxide or changed to less toxic compounds. Green liver concept of organic compounds could be easily classified as 3 processes such as transformation, conjugation, and localization (Kvesitadze and Kvesitadze, 2009). Phase I: transformation, toxic compounds would react with plant enzymes, which molecule could be changed the formation. Some organic compounds could be completely degraded in this process for example benzene, toluene, and xylene, and some compound might be reduced toxicities and accumulated in plant organelle such as trichloroethylene (TCE), trinitrotoluene (TNT), and polyaromatic hydrocarbons (PAHs). There are several reactions that could appear in this process. For example, the non-polar molecules, the polarity would be increased by addition of hydroxyl- group to the molecules by monooxygenase enzyme. Some organic compounds could be activated by addition of functional groups, which is suitable chemical group to conjugate with plant protein or glycosides.

Phase II: conjugation, after transformation process, activated molecule could combine to plant protein or glycosides. The process would decrease toxicities of molecule and stabilize them in a suitable form.

Phase III: localization, when toxic molecules were stabilized by conjugating with plant secretion compounds, the complex molecule would be localized in plant organelles such as cell wall, vacuole, etc. In soluble conjugated compounds, the compounds might be excreted out of the cells (Singh and Jain, 2003). Green liver concept had been presented in Fig 2.9.

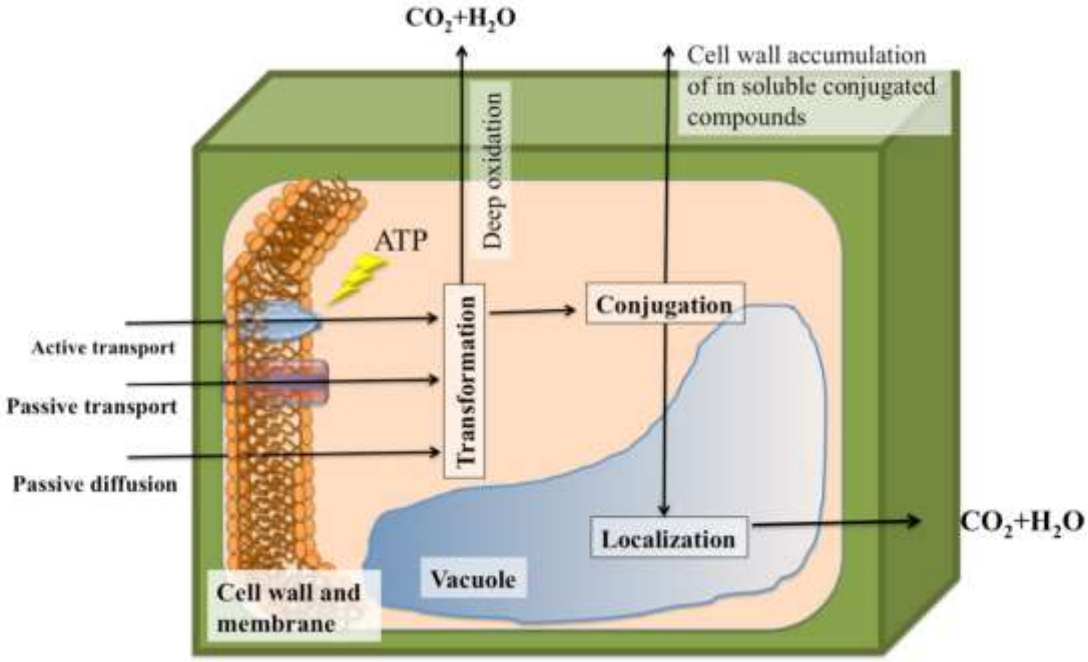


Figure 2.8 Principle of green liver concept.

Benzene uptake and transformation in plant

Uptake and distribution of benzene by plants

There are three important pathways of plant such as stomata, cuticles, and roots that plants use to uptake benzene, but the root of plants exposed to a little air because it stayed underground, so most of plants do not use roots to be the main pathway for benzene uptake. Stomata and cuticle are considered as main pathways for benzene uptake (Fig 2.10). Ugrekhelidze, *et al.* (1996) had studied on the 1-6^{14}C benzene uptake in 8 hours by 3 species of plants, which the experiment had compared between stomata pathway and cuticle pathway by the use of radioactive techniques. From this experiment, the abaxial side (stomata side) absorbs benzene more intensive than by adaxial side (cuticle side) in every treatment. So most of benzene had been reported that normally uptake by stomata side (Kvesitadze, *et al*., 2009). The result was shown in Table 2.9

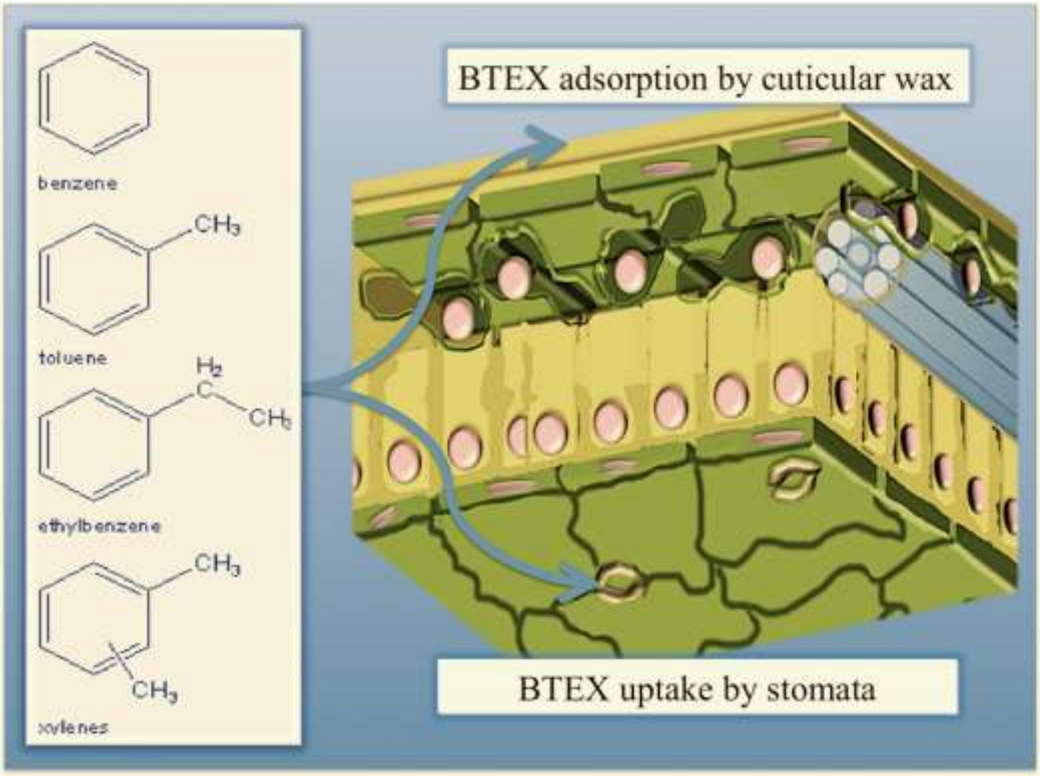


Figure 2.9 Gaseous BTEX uptake pathway in plant (stomata and cuticle).

Table 2.9 Total non-volatile benzene metabolites radioactivity from abaxial and adaxial side of plant after exposure to 0.1 mmol/L of gaseous benzene concentration; 1.76 MBq/mmol of radioactivity at 8 h exposure under light conditions (22-23ºC) (Ugrekhelidze, *et al*, 1996).

Species	Age	Absorption side	Stomata number per mm^2	Cuticle thickness (μm)	Total radioactive of non-volatile benzene metabolites (10^{-3} cpm/g fresh weight)
Acer campestre	young	abaxial	860	1.2	66.0
		adaxial		1.1	24.0
	old	abaxial	570	2.1	64.0
		adaxial		2.4	15.5
Viris vinifera	young	abaxial	70	1.0	42.5
		adaxial		1.0	19.0
	old	abaxial	40	1.6	49.0
		adaxial		1.7	17.5

In addition, this experiment also found that young leaves could uptake benzene higher than old leaves in 3 species of plants although young leaves have shown to be lower wax quantity than old leaf. This result, the composition of cuticle might effect on benzene uptake. Distribution of 4 species of plants had been also studied by a radioactive method. Cytosol had been found as the most important fraction in every species that contains the most percentage of total radio activities. Also, chloroplast had been also shown that contain high percentage of total radio activities as well. The result suggested that benzene and benzene metabolites could distribute well and accumulate in these 2 factions of plant cells (Table 2.10).

Table 2.10 Distribution of benzene and benzene metabolite intracellular organelles of plant leaf after exposure to 0.1 mmol L^{-1} of gaseous benzene concentration; 1.76 MBq $mmol^{-1}$ of radioactivity at 7 h exposure under light (22-23^{o}C) (Ugrekhelidze, *et al*, 1996).

Cellular organelles	**Percentage of total radioactivity**			
	Acer compestre	***Malus domestica***	***Vitis vinifera***	***Spinacia oleracea***
Nuclei and cell walls	5.2	7.3	4.1	6.0
Chloroplasts	33.6	29.8	28.8	30.7
Mitochondria	10.7	11.2	14.6	12.5
Microsomes	4.0	5.3	2.5	3.3
Cytosol	46.5	46.4	5.0	47.5

Benzene degradation by plants

Because benzene is an organic molecule, mechanism of benzene in plants is possible involved organic acids and amino acids from benzene degradation. Radioactive techniques had been used to analyze percent of total radioactivity of 1-6^{14}C benzene metabolites such as organic acids and amino acids in spinach leaves with 72 h 1-6^{14}C benzene exposure. 84% were found as organic acids, and 16% were as amino acids. In organic acids analysis, 37% and 24% were muconic acid and fumalic acid, respectively. In amino acid analysis, tyrosine was found as the main benzene metabolize about 34%, and 26% of phenylalanine had been found as second amino acid. Table 2.11 shows the percent of total radioactivity between organic acid and amino acid in spinach leaves after 72 h exposure.

For benzene transformation enzymes identification, 8-Oxyquinoline was known that is inactivate all the heavy metal enzymes, and sodium diethyldithiocarbamate suppresses the activity of copper-containing enzymes (Sato, 1966). Both inhibitor had been studied the relationship between the total radioactivity of nonvolatile metabolites and the present of inhibitors in spinach chloroplasts with 3 h of benzene exposure. The result was shown in Table 2.12.

Table 2.11 Non-volatile benzene and toluene metabolites, low molecular weight compounds, in plant after exposure to 0.2 mmol L^{-1} of gaseous benzene concentration; 1.76 MBq $mmol^{-1}$ of radioactivity in benzene and 1.5 MBq $mmol^{-1}$ of radioactivity in toluene at 72 h exposure under light conditions (22-26^{o}C) (Ugrekhelidze, *et al*, 1996).

Substrate	**Percentage of total radioactivity**		**Distribution of radioactivity in percentage**	
	Organic acid	**Amino acid**	**Organic acid**	**Amino acid**
[1-6 14C]Benzene	84	16	Muconic, 37.2 Fumaric, 24.4 Succinic, 12.5 Malic, 9.6 Oxalic, 9.1 X, 7.2	Tyrosine, 33.8 Phenylalanine, 25.8 Glycine, 16.2 Aspartic acid, 11.3 X1, 7.4 X2, 5.5
[1 14C]Toluene	79	21	Fumaric, 21.7 Malic, 18.9 Oxalic, 9.5 Succinic, 8.3 X1, 33.5 X2, 8.1	Tyrosine, 29.7 Aspartic acid, 26.0 Alpha-alanine, 14.8 Valine, 14.4 X, 15.1

Table 2.12 Transformation of benzene by spinach chloroplasts (Ugrekhelidze, *et al*, 1996).

Chlroplasts with ^{14}C benzene	**Total radioactivity of non-volatile benzene metabolites (10^{-3} cpm g^{-1} fresh weight)**	
	Dark	**Light**
Without additives	5.3	9.4
8-Oxyquinoline (10^{-3}M)	>0.1	0.5
α, α'-Dipyridyl (10^{-3}M)	4.7	7.8
Sodium diethyldithiocarbamate (10^{-3}M)	>0.1	0.8

From Table 2.12, the present of 8-Oxyquinoline and sodium diethyldithiocarbamate could inhibit benzene transformation enzymes both under light and dark conditions, but a little radioactive could be reduced by α,α'-dipyridyl that is the iron enzymes inhibitor. This experiment concluded that the benzene transformation enzyme contain copper as main component. Phenoloxidases and ascorbate oxidase that are copper-containing proteins of chloroplasts had been considered to be benzene transformation enzyme. Spinach chloroplasts possess less active ascorbate oxidase than O-diphenoloxidase (Sechneska, *et al*., 1968). So phenoloxidases might be the most important enzymes for benzene transformation. In addition, the interesting result is that NADH and NADPH could enhance benzene mechanism and transformation (Ugrekhelidze, *et al*, 1997). NADH and NADPH show the general reaction that could send $2e^-$ to P450 monooxygenase, and this enzyme contain the function of oxygen molecule activation to combine with hydrophobic molecule. So it is possible that NADH and NADPH can activate benzene metabolism and its transformation. The result was shown in Table 2.13.

Table 2.13 Transformation of benzene by spinach leaf enzyme preparation (Ugrekhelidze, *et al*, 1996).

Enzyme preparation with 14C benzene	**Total radioactivity of non-volatile benzene metabolites (10^{-3} cpm g^{-1} fresh weight)**	**C^{14} phenol formed (10^{-3} cpm g^{-1} fresh weight)**	**inhibition (-) and activation (+) %**
Without additives	650	0	0
8-Oxyquinoline (10^{-3}M)	67	0	-89.7
α, α'-Dipyridyl (10^{-3}M)	538	0	-17.3
Sodium diethyldithiocarbamate (10^{-3}M)	83	0	-87.3
NADH	4660	1.3	+716.9
NADPH	4110	1.2	+632.3

In 2009, Kvesitadze and Kvesitadze (2009) had reported the mechanism of organic molecule in plants. Benzene could be degraded by two general plant enzymes such as phenoloxidases and P450 monooxygenase. P450 monooxygenase can generate hydroxyl group on the benzene molecules. In this pathway NADH and NADPH provide $2e^-$ for cytochrome b_5 reductase activation which b_5 allows $2e^-$ to stimulate P450 monooxygenase. This pathway is shown in Figure 2.11.

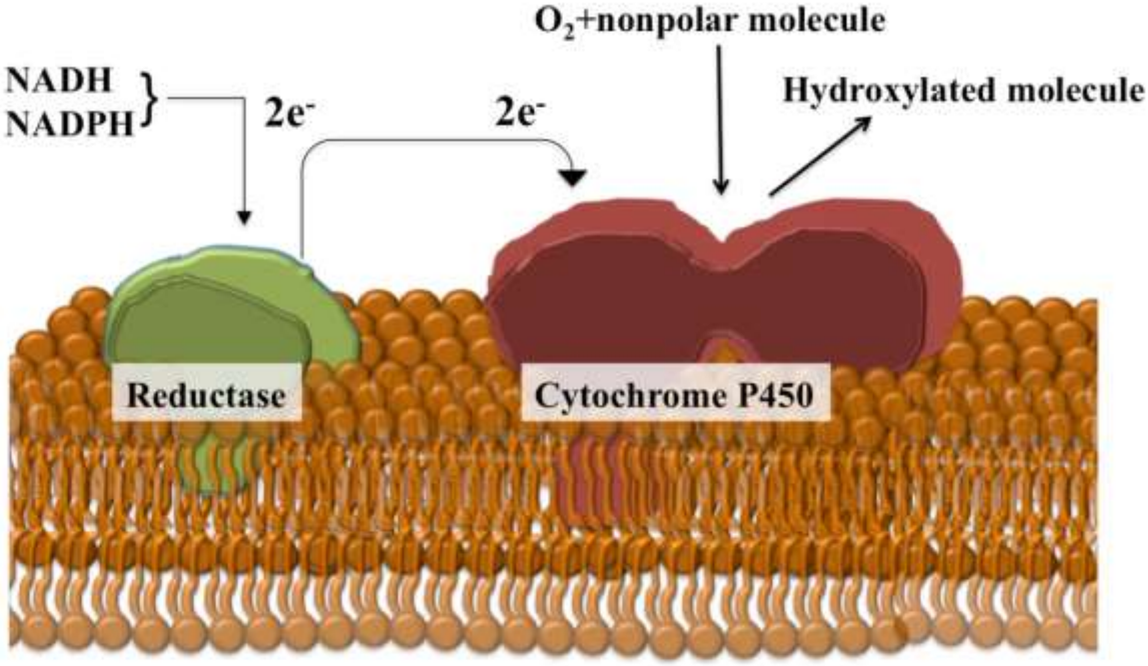


Figure 2.10 P450 monooxygenase function for hydroxyl group addition on xenobiotic molecules.

Benzene could be changed to phenol, and phenol would be added hydroxyl groups by P450 monooxygenase again and changed to catechol. Not only P450 monooxygenase but also phenoloxidases could generate the molecules of catechol. Oxygen active species are also generated by the reaction of o-diphenoloxidase and o-diphenol. This oxygen active species could directly combine with benzene or phenol molecule and create catechol molecule. Figure 2.12, phenoloxidase function for oxygen active species generation and benzene metabolism by oxygen active species.

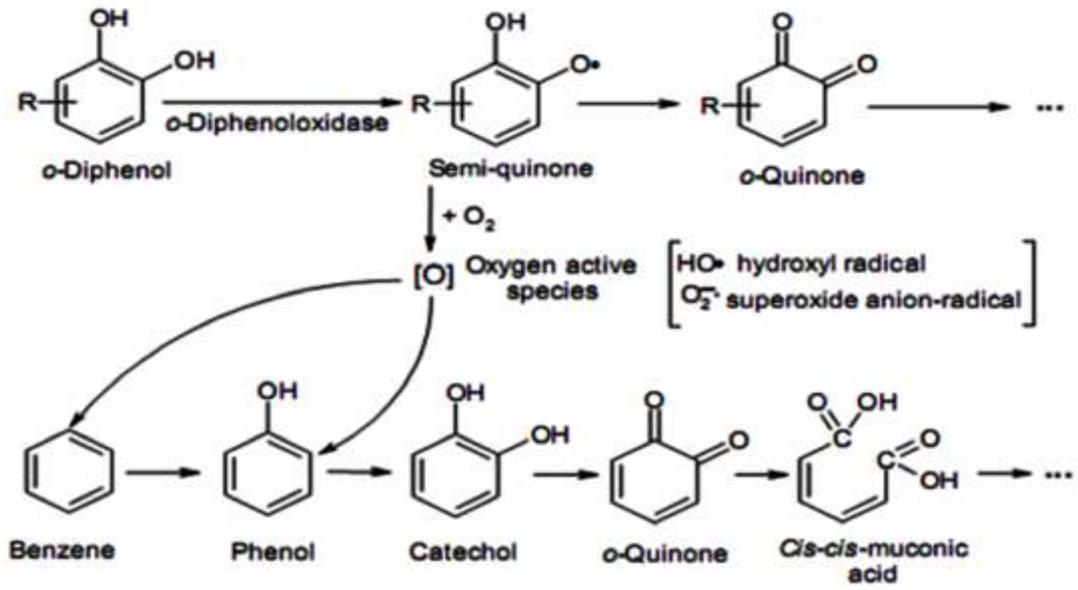


Figure 2.11 Phenoloxidase function for oxygen active species generation and benzene metabolism by oxygen active species.

Benzene metabolism is concluded that after catechol molecule is generated, it could be changed to o-quinone molecule. This o-quinone is normally known as unstable molecule, which could be braked easily the ring structure and changed to muconic acid. This muconic acid, which is general molecule in living cells, is produced from o-quinone ring cleavage. This molecule contains less toxic and can be degraded to CO_2 by TCA cycle (Fig 2.13). The use of benzene as carbon and energy sources had been firstly proposed.

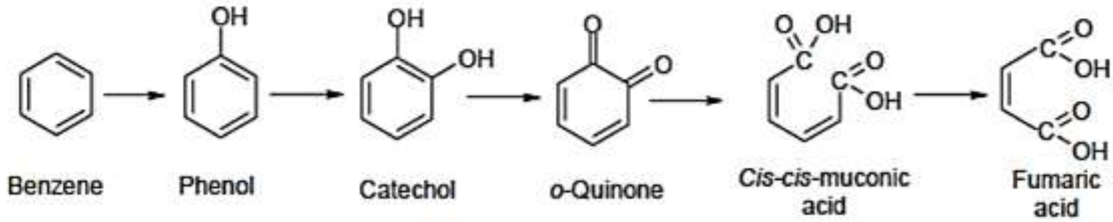


Figure 2.12 Benzene metabolisms in plant cell (Kvesitadze and Kvesitadze, 2009).

Plants application for benzene removal

In 1989, 10 species of plant such as *Gerbera jamesonii*, *Chrysanthemum morifolium, Hedera helix, Sansevieria laurentii*, *Dracaena deremensis warneckei*, *Spathiphyllum, Aglaonema*, *Dracaena marginata*, *Chamaedorea seifrizii*, and *Dracaena deremensis* were presented high benzene removal efficiency at initial benzene concentration of 20 ppm (Wolverton, et al., 1989). *Gerbera jamesonii* and *Chrysanthemum morifolium* can uptake highest benzene about 23.5 μg cm^{-2} and 18.2 μg cm^{-2}, respectively. In 2004, Orwell, et al. (2004) screened 7 ornamental plants to remove benzene such as *Dracaena* "Janet Craig", *Epipremnum aureum, Dracaena marginata, Schefflera* "Amate", *Spathiphyllum* "Petite", *Spathiphyllum* "Sensation", *Howea forsteriana* at initial concentration of 25-50 ppm. The results showed that *Dracaena marginata* can uptake highest benzene about 23.23 µg cm^{-2}. However the similar species was found only 4 µg/cm^2 of benzene removal in Wolverton study. When *Dracaena* "Janet Craig" in Wolverton study can remove 1.7 µg cm^{-2}, 13 µg cm^{-2} benzene removals was found in Ralph study. The results suggested that growing condition may effect on benzene removal efficiency. In 2007, Liu, *et al*. (2007) had studied 72 species for benzene removal by continuous system at 150 ppb of initial benzene concentrations. High benzene removal efficiency species were shown in Table 2.14.

Table 2.14 Mean benzene removal efficiency in each plant (µg m^{-2} day^{-1}) by dynamic system experiment.

Plant species	**µg m^{-2} day^{-1}**
Crassula portulacea	724.9
Hydrangea macrophylla	293.7
Cymbidium golden	267.4
Ficus microcarpa	255.5
Dendranthema morifolium	204.9
Citrus medica	166.7
Dieffenbachia amoena	115.2
Spathiphyllum supreme	106.9
Nephrolepis exaltata	73.5
Dracaena deremensis	59

Tarran, *et al.* (2007) had showed that at the first cycle benzene exposure, plant could uptake toxic molecules slower than other benzene exposure cycles in every plant in *Zamioculcas* and *Aglaonema*. This tendency suggests that plant required the time for its adaptation. The result of this experiment was shown in Figure 2.14.

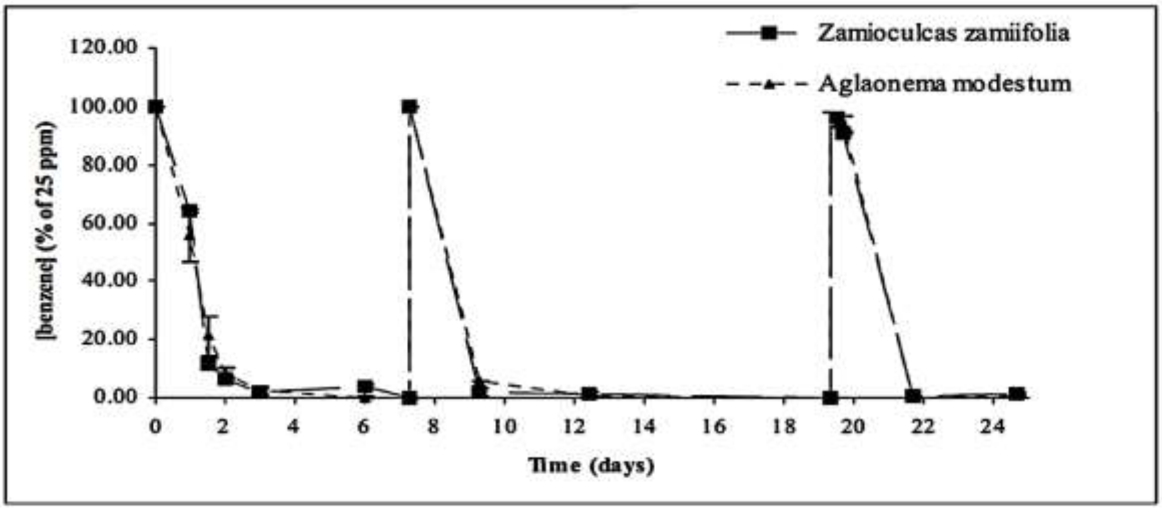


Figure 2.13 Removal of benzene from test-chamber air by potted *Zamioculcas zamiifolia* and *Aglaonema modestum*, challenged with three consecutive doses of 25 ppm benzene.

The similar result had been found in 2001. Wood, *et al*. (2001) studied on the benzene removal efficiency of *Spathyphyllum var Petite* plants when was exposure with 25-30 ppm of benzene in both light and dark. In light condition, the tendency of benzene removal efficiency was similar to Jane Tarran's study. First cycle of benzene exposure, plant required time to adaptation. In addition, slower benzene removal tendency was found in dark condition. The result was shown in Figure 2.15. Light might be important factor for benzene removal in plant. 2 reasons that might be possible to explain this event is that NADH and NADPH, electron donator for benzene degrading enzyme, are required photosynthesis to generate and the open of stomata under light condition could promote gaseous uptake by plant.

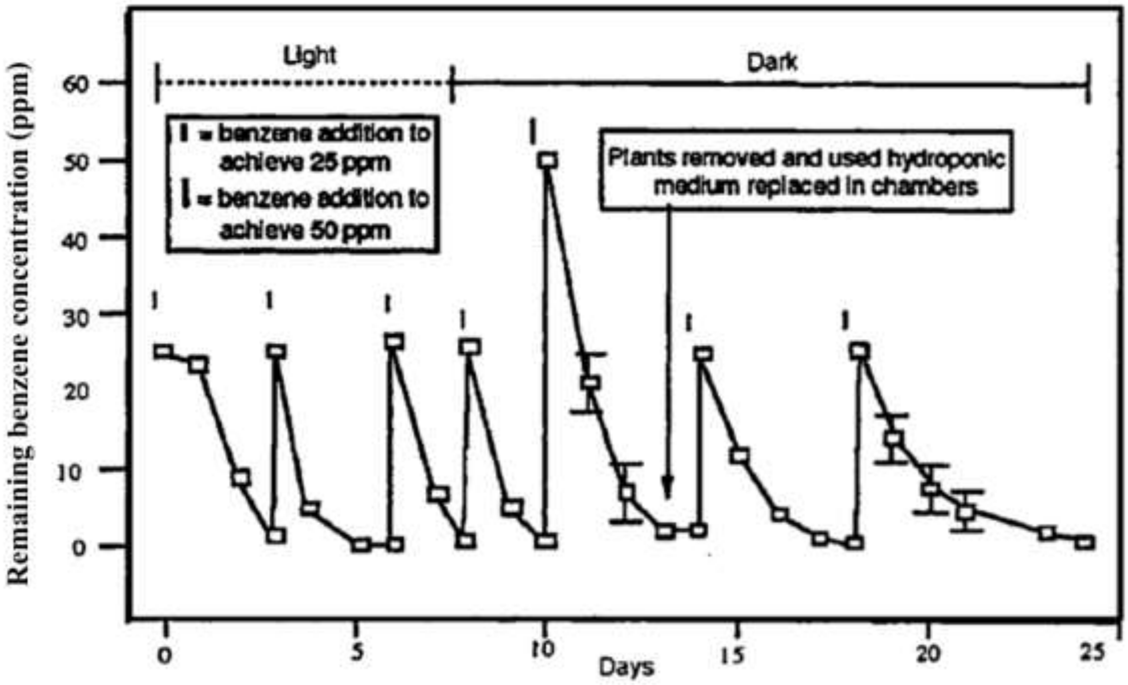


Figure 2.14 Benzene levels in air of test chambers containing Spathyphyllum var Petite plants maintained in hydroponic medium.

Possibility of benzene adsorption by plant leaf wax

Principle of plant leaf wax

Almost hydrophobic molecule on plant leaf was found as glycerol lipids and fatty acids. Glycerol lipids that have been known as main composition of wax containing one glycerol and 1-3 fatty acids. In monoglycerides, glycerol had been esterified by 1 fatty acid in *sn-1* or-*2* positions. Diglycerides are the main composition on the cell membrane however polar head with phosphate group was often occurred. Finally, triglycerides are the common lipid form in living cells. In fatty acid composition, R-COOH is the common form of organic acid and fatty acids. R is identified to alkyl or alkane groups. In general, fatty acids and organic acids were separated by the alkyl or alkane groups if alkyl or alkane groups contain less than 4 carbon atom contents, which will be classified as only organic acids.

Benzene adsorption and accumulation by plant cuticle

The 1-6^{14}C benzene uptake pathway in 3 species of plants by the use of radioactive technique was studied (Ugrekhelidze, *et al.*, 1996). Plant can uptake gaseous benzene though stomata and cuticle on the surface of leaf. In addition, in dark condition, plant can still grow and uptake benzene well that should be the benzene uptake by only waxes when stomata was closed under dark condition (Orwell, *et al.*, 2004). The accumulation of benzene in wax layer of plant leaf had been found in many researches (Gorna-Binkul, *et al*., 1996; Slaski, *et al*., 2000; Tsiros, *et al*., 1999; Collins, *et al*., 2000; Poborski, 1988; Reiderer, 1990; Kylin, *et al*., 1994). In 1996, Gorna-Binkul, *et al*. (1996) found the benzene and it derivatives accumulate on orange shell, and parsley. Black berry, apple, and cucumber were also investigated benzene accumulation. The result found that black berry and apple can well accumulate benzene (Collins, *et al*., 2000). Not only benzene but also poly-aromatic-hydrocarbon (PAHs) was also reported the accumulation in many part of plant that grown in contaminated environment (Slaski, *et al*., 2000). Benzene, contains 2.13 of log K_{OW}, can diffused rapidly into the plant (Kamath, *et al*., 2004). Ugrekhelidze (1996) observed also the ^{14}C-benzene and non-volatile benzene metabolites both old and young plant leaves. The result suggested that although high quantity of cuticle was found in old leaf, young leaf have more benzene and it metabolites accumulation intensity than old leaf. This result, the composition of wax may be a main effecting factor for VOCs adsorption.

Chapter 3 Materials and Methods

Apparatus

1. 2 and 4 decimal analytical balance

2 Hot air oven

3 Glass desiccator

4 Gas chromatography with flame ionization detector, GC-FID (Bruker, 400GC-series)

5 Hot plate

6 Volumetric flask

7 Test tube

8 Aluminium foil and plate

9 Paraffin tape

10 Gas tight syringe

11 Light microscope

12 Chlorophyll fluorometer, kings lynn, England

13 Watman filters paper No. 2

14 GC-MS, DB5 capillary column

15 FT-IR

16 Vacuum pump model VE115N with 2.0 CFM free air displacement

17 Retsch ultra centrifugal mill

Material and chemical reagent

1 99.8% Benzene, panreac, E.U.

2 Hexane

3 Ethyl alcohol

4 BSTFA with 1% TMCS

Plant preparation

Ornamental plants such as *Chamaedorea seifrizii, Scindapsus aureus, Sansevieria trifasciata, Philodendron domesticum, Ixoraebarbata craib, Monster acuminate, Epipremnum aureum,* and *Dracaena sanderiana* were purchased from plant shops in Thailand. Similar leaf area appearance in each species was chosen. The roots had been covered by wet tissue paper in every species, and then the roots had been wrapped again by aluminum foil. These plants after preparation had been used to study benzene absorption in static system.

Plant leaves material preparation

Twenty-one plants such as *Homalomena rubescens, Citrus hystrix*, *Musa paradisiaca, Mangifera indica*, *Catura metet*, *Lagerstroemia inermis*, *Cananga odorata*, *Cassia siamea, Bougain villea, Litchi chinensis, Coccinia grandis*, *Dieffenbachia picta*, *Attacus atlas, Polyalthia longifolia, Acrostichum aureum, Ficus religiosa, Alstonia scholaris, Anthurium andraeanum, Plerocarpus Indicus, Lagerstroemia macrocarpa,* and *Dracaena sanderiana* were purchased from plant shops in Thailand. Leaf of each plant was cut and dried under 60 ^{o}C for 2 days. Dry leaf of plants had been powdered by Retsch ultra centrifugal mill with 14,000 cycles min^{-1} and dried again under 60^{o}C for 2 days in oven. Leave materials had been collected in dry bottle to control the humidity. These materials had been used in 3 kinds of experiment such as static adsorption, dynamic adsorption and biofilter. In static system, 0.2 g of each plant leave material was put in fumigator for passive benzene uptake. In dymamic system, selected plant leave materials were immobilized on glass bead by cassava powder glue. Plant leave materials-immobilized glass bead were oven under 60 ^{o}C for 2 days. In biofilter, *A. aureum* and *A. scholaris* leave materials immobilized glass bead were applied as a media for *P. putida*. The UV was applied for sterilization of *A. aureum* and *A. scholaris* leave materials immobilized glass bead.

Crude wax extraction and crude wax quantity analysis

One g of plant leave and leave materials had been immersed in 50 mL of hexane at 4 ^{O}C over night for extraction of hydrophobic wax. Extracted solution was filtrated by Whatman number 2-filtration paper with 8 µm of pore size. After filtration method, the wax extracted solution was put in ceramic cup, and nitrogen was applied to flow though the sample for hexane evaporation on ceramic cup. Weight of crude wax was calculated by the different of ceramic cup weight before and after wax immobilization. In this experiment, 4 decimals balance had been used.

Microorganism preparation

Freeze dry *Pseudomonas putida* TISTR1522 was purchased from Bangkok Microbiological Resources Centre (MIRCEN), is the main service collection in Thailand. 25 mL NB nutrient was used to grown this stain, and room temperature was applied for *P. putida* TISTR1522 incubration overnight by 150 cycles min^{-1}. This microorganism had been inoculated in biofilter.

Nutrient for microorganism culture

Lysogeny Broth (LB) was used as enriched medium, and minimum medium was prepared from 20 mg $CoCl_2•6H_2O$, 30 mg H_3BO_3, 10 mg $ZnSO_4•7H_2O$, 1 mg $CuCl_2•2H_2O$, 2 mg $NiCl_2•6H_2O$, 3 mg $NaMoO_4•2H_2O$, 10 mg $FeSO_4•7H_2O$, 2.6 mg $MnSO_4•H_2O$, and 1000mL ultrapure water (Kragelund and Nybroe, 1994). Before nutrient injection, 121 ^{O}C and 20 minute with pressure of 15-pound $inch^{-2}$ was applied for autoclave-sterilized nutrient.

Fumigatory chamber preparation

Glass desiccators, which contain 15.6 L of volume, were used to fumigate plants and it materials with benzene. The static system was closed and sealed by paraffin tape. Three aluminum plates were also put in the chamber to help benzene homogenous. The special lid of the chamber that had been specifically designed contained 2 rubber septums for injection and sampling. The desiccators had been applied as a benzene exposure system in every static experiment. Living plants and plant leave materials were exposure with benzene in this chamber.

Benzene removal in a static system by living plants

Eight ornamental plants, which contain leaf area more than 130 cm^2, were placed in designed desiccators under normal indoor conditions (3 repetition chambers in each treatment). Desiccators were closed and sealed by paraffin tape at temperature (~32 ^oC) and pressure (~760 mmHg). These temperature and pressure were used to calculate mole concentration (M_c) by Eq 3.1.

$$\mathrm{Mc} = 24.47 \times \frac{760}{P} \times \frac{T + 273.15}{298.15} \qquad \text{(Eq 3.1)}$$

$$20\ ppm = 10^6 \times \frac{W}{M_w} \times \frac{M_c}{V} \qquad \text{(Eq 3.2)}$$

$$\rho = \frac{W}{V_b} \qquad \text{(Eq 3.3)}$$

M_c is mole concentration. P (mmHg) and T (°C) are pressure and temperature, respectively. 20 ppm was specified. M_W (molecular weight of benzene) and V (the volume of the chamber (L)) were substituted to calculate W (benzene weight (g)) by Eq 3.2. ρ (benzene density (g/ml)) and weight of benzene were used to predict V_b (benzene volume (mL)) by Eq 3.3. 99.8% benzene, which was purchased from Panreac (made in E.U.), should be injected at 1±0.1µL for 18±2 ppm of benzene concentration. From the system optimization, four hours were required for benzene equilibrium in this system, so the samples in every experiment were collected after 4 h. Remaining benzene had been collected in 4, 6, 8, 24, 48, and 72 h and analyze by gas chromatography with flame ionization detector, GC-FID (Bruker, 400 GC-series)

Benzene removal by living plants under dark and light conditions

Same size of *D. sanderiana* had been grown and exposure with gaseous benzene under light and dark conditions. In light condition, *D. sanderiana* was grown under 24 h normal lamplight. In dark condition, black paper was used to cover the benzene exposure chamber, creating 24 h dark conditions. Liquid benzene was injected in to the chamber follow on the protocol of benzene removal in static system by plant. Benzene uptake in both conditions had been analyzed and compared in a long-time exposure (4 cycles) to make sure that this technology could be sustainable benzene removal method. Seven days were applied in each cycle, and 20 ppm of benzene was started in each cycle. 0.3 mL of gaseous benzene in the chamber was collected by gas tight syringe and measured by gas chromatography with flame ionization detector, GC-FID (Bruker, 400GC-series). Three replication and 3 samples in each treatment were required. Remaining benzene concentration had been observed in day 1, 2, 3, 5, 7 of each cycle.

Benzene removal by wax of plant

A 130 cm^2 sized of leaves of *D. sanderiana* were cut and collected, and crude wax was extracted by hexane. The leaves were immersed in 200 mL hexane overnight at 4^{o}C of temperature. Crude wax solution was transferred in to a 130 cm^2 aluminum plate that was the similar size of leaves of *D. sanderiana*, and hexane was evaporated by the flowing of nitrogen. The aluminum plate with crude wax was used to study the benzene uptake by wax. Crude wax immobilized on aluminum plate was fumigated with 20 ppm of benzene in a chamber that follow on the method in benzene removal by plant, and 0.3 ml of gas was collected in each chamber at temperature (~32 ^{o}C) and pressure (~760mmHg). There are 3 replications and 3 samples in each treatment. The samples were collected at 24, 48, 72, 120, and 168 h. The result had been used to calculate the benzene adsorption by crude wax and investigate benzene uptake pathways.

Stomata observation

Replica techniques, nail varnish could be applied to copy the pattern of stomata on the leaves. Nail varnish was painted on the stomata side on leaf surface, and dry nail varnish after pattern copying was carefully keep out. A light microscope with 100 x was applied to observe the structure of stomata and count number of stomata that occurred on dry nail varnish. The number of stomata had been used to find the relation with benzene removal efficiency, and structure of stomata had been observed from growing *D. sanderiana* under light and dark conditions.

Benzene removal in a static system by plant leaf materials

Plant leaf materials, each containing about 0.2 g, were placed in modified desiccators with injection and sampling pot under indoor conditions (3 repetition chambers in each treatment). Desiccators were closed and sealed by parafilm at temperature (~32 ^{o}C) and pressure (~760 mmHg). In this experiment, the equation from benzene removal by plant under static system had been applied. 20 ppm of benzene was used to be initial benzene concentration, and the remaining benzene concentration had been measured in day 1, 2, 3 because from preliminary study, benzene uptake by cuticle had been saturated normally in 3 days. The experiment had been done in 21 materials from plant leaves. The result was analyzed to select the high benzene adsorption capacity materials.

Benzene adsorption by a dynamic system

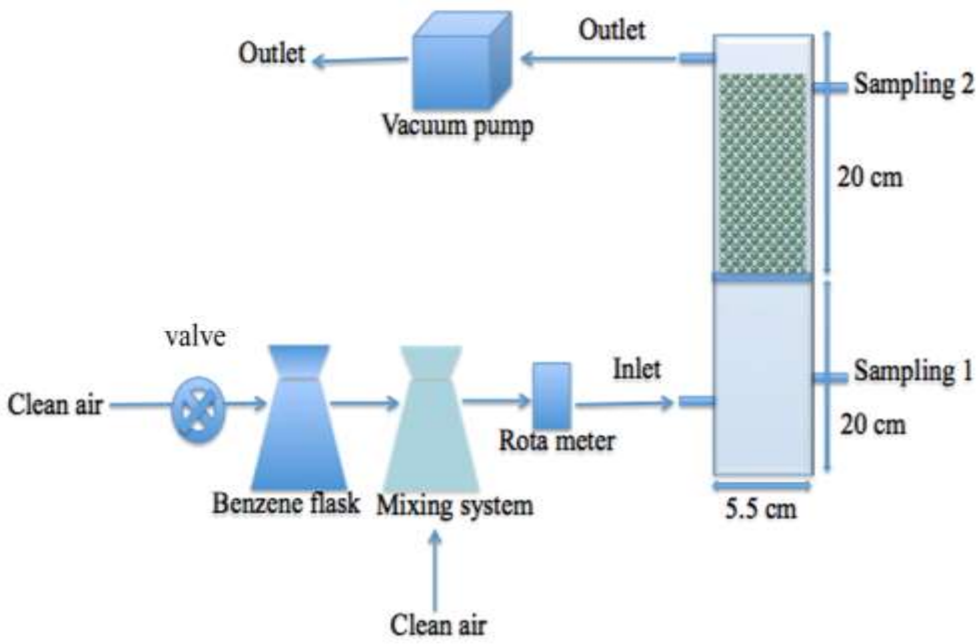


Figure 3.1 Benzene adsorption system.

Vacuum pump model VE115N with 2.0 CFM free air displacement was used to flow clean air. Clean air was flow though controlled valve and was pumped though benzene flask that contain 50 mL benzene. Contaminated air was diluted with clean air in mixing flask, and Rotameter was applied for benzene contaminated airflow rate regulation. Initial waste gas of benzene (~55 ppm) was flowed into glass column, which have 40 cm height and 5.5 cm diameter. Above column bottom 20 cm, glass pore disc was used to create layer of adsorbent. Contaminated gas was flowed though absorbent layer, and out from the system. Before waste gas was flow out of the system, activated carbon was used to treat completely waste gas. Sampling pot 1 and 2 on the column was used for sample collection (Fig 3.1). The continuous study was followed by condition in Table 3.1.

Table 3.1 Continuous system conditions in each selected plant leaf materials.

	D.sanderiana	***D.picta***	***F.religioza***	***L.macrocarpa***	***A.scholaris***	***A.aureum***
Weight of leaf (g)	15	15	15	15	15	15
Flow rate (L min^{-1})			0.03-0.05			
Filter volume (mL)	93.75	103.5	108.75	120	120	144
Filter Depth (cm)	15.79	18.95	15.79	12.63	12.63	15.16
EBRT (min)	3.125	3.45	3.625	4	4	4.8
Initial benzene concentration (ppm)				55		

Sampling pot 1 was collected to measure benzene concentration of inlet, and sampling pot 2 was used to analyze outlet waste gas. Plant leaf materials-immobilized glass bead was used to be the treatment. Starch glue-immobilized glass bead and only glass bead were used as control.

Benzene adsorption mechanism

Hexane was applied to desorb benzene from absorbent after capacity saturation of adsorbent was occurred. 1 g of every plant leaf materials was immerged in 50 mL hexane. Benzene desorption from plant leaf materials had been analyzed by GC-FID (Bruker, 400GC-series) with 250 ^{o}C Injector, 200 ^{o}C Detector and 100 ^{o}C Oven and split mode 1:10, and percentage benzene desorption were calculated. In surface functional group observation both before and after benzene adsorption, the samples of every plant leaf materials were analyzed by Fourier transform infrared spectrometer (FT-IR), and Perkin-Elmer spectrum one. The KBr disc technique was used to carry spectra of absorbents. In BET porosity analysis, Surface porosity of each material was measured by surface area and porosity analyzer model Autosorb-1 (Quantachrome).

Gas analysis

Every day, samples of 0.3 mL waste gas were collected from chamber in chamber. GC (gas chromatography, 400GC-series bruker) was used to analyze benzene concentration. Benzene was measured by 105-meter length and 0.53 mm ID column. Diphenyl/Dimethyl polysiloxane phase were filled as a stationary phase. N_2 was used as a carrier gas. FID cylindrical electrode detector with detection limits of 3×10^{-12} g s^{-1} and dynamic range of 10^7 were used. The benzene uptake by biomaterial from plant was calculated follow the equations:

$$\Delta ppm = ppm_{control} - ppm_{treatment} \quad \text{(Eq 3.10)}$$

$$W = \Delta ppm \times \frac{V}{M_c} \times \frac{M_w}{10^6} \quad \text{(Eq 3.11)}$$

$ppm_{control}$ and $ppm_{treatment}$ are the remaining benzene concentration in control and treatment systems, respectively. The differences between $ppm_{control}$ and $ppm_{treatment}$ were used as benzene uptake by biomaterial from plants (Δppm), and benzene uptake by plants was calculated to the weight (W) of benzene uptake by biomaterial from plants (g) following

Eqs 3.10-3.11. V (L) is the volume of the system. M_c is mole concentration of benzene. M_W is molecular weight of benzene. Weight of benzene uptake per weight of plant leaf material was reported.

Statistical analysis

Descriptive statistic that was used usually in this experiment was mean and standard deviation. One Way ANOVA with 95% confident level was applied to compare mean of each treatment, and Duncan's multiple range tests are used to classify significantly the group of all data in this experiment. Correlation coefficient was used to analyse all relationship. Statistic package for social science (SPSS) version 19 was the program to analyse the data in this study.

Chapter 4 Result and discussion

Benzene removal by living plants

Benzene phytoremediation efficiency

Although phytoremediation is not novel process to clean up the environment, there are not many examples of the use of plants to clean up air pollution. Gaseous benzene distribution and degradation *in planta* was first investigated in 1996. Radioactive measurements suggested that plants can uptake benzene though both stomata and cuticle, and that benzene absorbs easily into cells. Passive diffusion is known to be the diffusion mechanism in this case. After benzene diffuses into plant cells, cytochrome P450 mono-/di-oxygenases and phenoloxidases could transform benzene to benzene metabolites such as catechol and phenol. Benzene may be completely degraded to carbon dioxide. However approximately 95% is converted to organic and amino acids of the TCA cycle. Most benzene and benzene metabolites accumulates in the cytosol (Ugrekhelidze, *et al*, 1997; Kvesitadze, *et al*., 2009). These experiments demonstrate that benzene can be taken up and transformed by plants which can be applied to develop highly effective methods for indoor gaseous benzene treatment. There are many experiments showing efficient benzene removal from air by plants, the first being the screening of 50 plant species for removal of 20 ppm benzene in a static system in 1989. The NASA scientists found 10 species to remediate benzene with particularly high efficiency, including *Gerbera jamesonii, Chrysanthemum morifolium, Hedera helix, Sansevieria laurentii, Dracaena deremensis warneckei, Spathiphyllum, Aglaonema, Dracaena marginata, Chamaedorea seifrizii,* and *Dracaena deremensis* (Wolverton, *et al*., 1989). In 2004, Orwell, et al. then proposed the use of plants to remove benzene as a sustainable technology. In China, 72 plant species were screened for benzene removal in 2007. Ten species particularly efficient in benzene removal were identified in this screening (Liu, *et al*., 2007). In this study, 8 indoor ornamental plant species were exposed to 20 ppm benzene in a modified chamber for 72 h, and the remaining benzene in the experimental system was measured (Fig 4.1). The result shows that *D. sanderiana* has the highest benzene removal efficiency of the species investigated. Benzene uptake was 10.00±1.04 µmole of benzene at 72 h. Benzene uptake was also standardized by leaf area; standardized by leaf area, *D. sanderiana* was still the most efficient at benzene uptake, taking up 59.66±6.67 nmole cm^{-2} leaf area at 24 h of treatment and taking up 74.65±11.55 nmole cm^{-2} leaf area at 72 h of treatment. The benzene uptake of *S. trifasciata* increased with time, reaching

92.55±11.67 nmole cm^{-2} at 72 h. Orwell, *et al.* (2004) previously reported that *Dracaena* sp. can take up high amounts of benzene in 2004. The experiment had been done under 25-50 ppm of benzene, and benzene uptake by 7 plant species had been investigated. In this experiment *Dracaena marginata* took up 297 nmole cm^{-2} after 24 h. However, Wolverton, *et al*. (1989) reported benzene removal of only 51.2 nmole cm^{-2} by *D. marginata*, and 21.8 nmole cm^{-2} by *D. deremensis* after 24 h at 20 ppm of initial benzene concentration. In real world, benzene accumulation in ambient condition such as home or office can be around 0.0021 ppm. To remove this concentration of benzene within 24 h in the 6x4x3 m^3 room, only 1 living *D. sanderiana* plants should be enough (see appendices calculation data). The difference in benzene removal efficiency suggests that there are several factors affecting benzene uptake by plants, for example growth conditions, species of plant, etc. From previous experiment, not only benzene but also other VOCs such as xylene, toluene, and ethylbenzene can be also removed by plant. However following Fick's first law (Eq 4.1), molecular size of pollutant had been found as an important factor on phytoremediation of air pollution. Where J is the diffusion flux (mole m^{-2} s^{-1}), and D is the diffuse coefficient (m^2 s^{-1}), and $\emptyset$ is the concentration in dimensions. x (m) is position.

$$J = -D\frac{\partial \emptyset}{\partial x} \qquad \text{(Eq 4.1)}$$

Low molecular weight compounds, plant can uptake better than high molecular weight compounds, so benzene, which had lowest molecular weight when it is compared with other BTEX, should be uptake well by plant (Sriprapat and Thiravetyan, 2013).

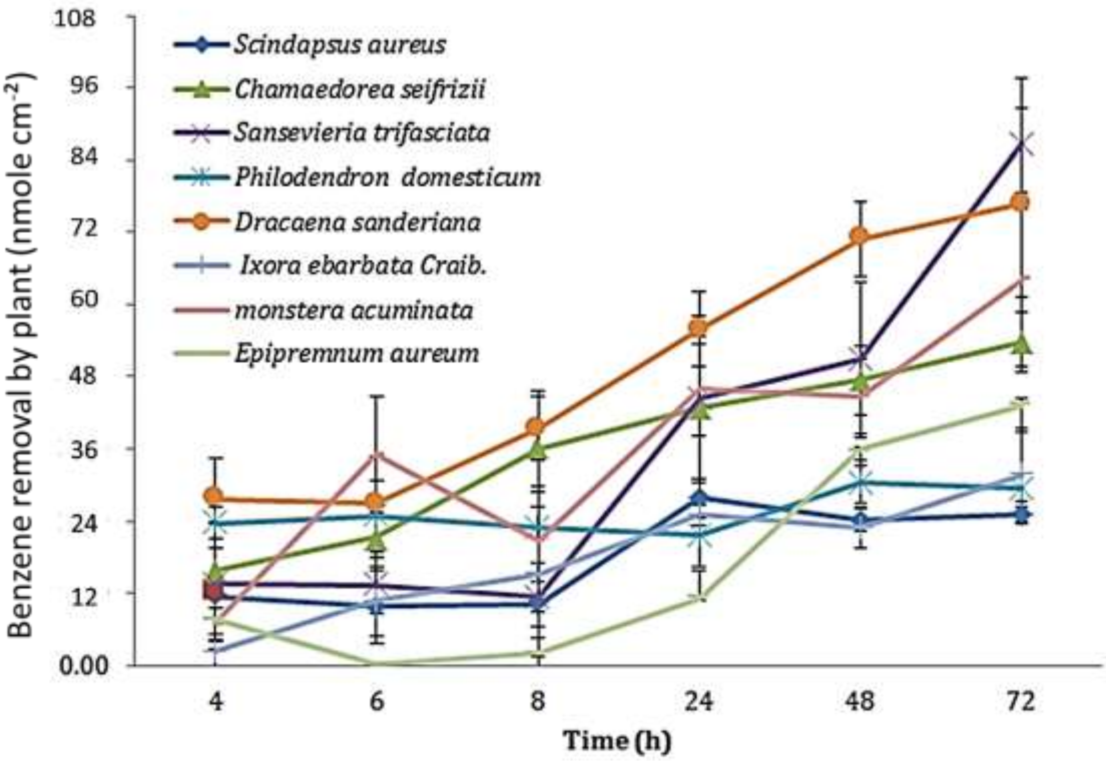


Figure 4.1 Benzene uptake by 8 ornamental plants

Stomata number, wax quantity, photosynthesis, and benzene removal efficiency in 8 ornamental plants

Factors which may affect benzene uptake efficiency were analyzed. Table 4.1 shows the number of stomata, amount of crude wax, photosynthesis rate, and the percentage of benzene removal after 72 h for each plant species investigated. *S. trifasciata* and *D. sanderiana,* the most efficient species at removing benzene, contain high quantities of crude wax. These results suggested that quantity of wax could affect benzene uptake efficiency. However correlation of wax quantity and benzene removal was investigated further. In *D. sanderiana*, a particularly high stomata density was also observed, while *S. trifasciata* had relatively low stomata density when compared with the other species, and so the relationship between stomata density and benzene uptake was not investigated further. Moreover, shape of stomata might also affect benzene uptake efficiency as well. Photosynthetic performance was measured as the ratio between variable fluorescence and maximal fluorescence (Fv/Fm) after 5 min in darkness. This represents the photochemical quenching capacity of photosystem II and operation of photosynthesis. No relationship between photosystem II activity and benzene uptake was found.

Table 4.1 Number of stomata, crude wax, photosynthesis, and percentage of benzene removal at 72 h of various plants.

Plant species	leaf area (cm^2)	Stomata number (Number of stomata cm^{-2})	Crude wax (µg/ leaf area cm^{-2})	Photosynthesis (Fv/Fm)	Range of % benzene removal at 72 hours
S. aureus	$149.67\pm25.5^{b,c}$	$3,750\pm901^{b,c}$	203.85 ± 5.44^{b}	0.81 ± 0.01^{c}	43-50
C. seifrizii	$131\pm30.05^{a,b}$	$5,917\pm382^{d}$	16.15 ± 0.00^{a}	0.79 ± 0.02^{c}	51-68
S. trifasciata	$110\pm10^{a,b}$	$1,167\pm382^{a}$	890 ± 162.09^{c}	$0.57\pm0.29^{a,b}$	54-77
P.domesticum	97.67 ± 25.42^{a}	$4,667\pm191^{c}$	33.46 ± 3.81^{a}	0.80 ± 0.00^{c}	50-62
D.sanderiana	180 ± 0.00^{c}	$10,583\pm1,134^{e}$	769.23 ± 0.00^{c}	0.45 ± 0.04^{a}	60-77
I. craib.	$131.67\pm25.07^{a,b}$	$18,444\pm509^{f}$	11.92 ± 1.63^{a}	$0.74\pm0.01^{b,c}$	65-72
M. acuminata	240 ± 36.06^{d}	$6,083\pm505^{d}$	5.77 ± 0.54^{a}	0.84 ± 0.05^{c}	52-62
E. aureum	$128.67\pm24.85^{a,b}$	$3,750\pm250^{b,c}$	230.77 ± 0.00^{b}	0.78 ± 0.03^{c}	56-59

Data are list as average ± SD for three replications. Duncan's multiple range tests with 95% confident level was used to classify the group of data.

Benzene removal by living plant under light and dark conditions

To study the sustainability of this technology and the effect of light, *D. sanderiana*, which is high benzene removal plant from screening study, had been grown under 24 h light and dark conditions and benzene removal had been analyzed. For sustainability of technology, the experiment had been designed as 4 cycles with 20 ppm of initial benzene concentration in each cycle. *D. sanderiana* growing under 24 h light condition clearly showed higher benzene uptake efficiency than growing under 24 h dark conditions at 2nd, 3rd, and 4th cycles (Fig 4.2). This result similar to Wood's study in 2001 that *Anubias barteri*, exposed to 25 ppm of benzene in light, removed benzene faster than in the dark. Sustainability of phytoremediation of gaseous benzene had been reported in this experiment (Wood, *et al*., 2001). In 2007, sustained benzene uptake by *Zamioculcas* and *Aglaonema* had been also reported (Tarran, *et al*., 2007). For benzene degradation by plant cytochrome P450 enzymes, electron donors such as NADH and NADPH are normally required (Kvesitadze, *et al*., 2009). These 2 electron donor molecules could be generated by photosynthesis of plant that light is commonly required. The exposure of 24 h light might increase quantity of electron donor and improve benzene removal. In addition, stomata had been considered as important for gaseous benzene uptake (Ugrekhelidze *et al*., 1996), and in most plant species stomata open under light conditions, so stomata of *D. sanderiana* in each cycle had been observed by general light microscope. At the 2^{nd}, 3^{rd}, and 4^{th} cycles under 24 h dark conditions, the closed stomata were observed (Fig 4.3). The result suggests that benzene could be removed better under light conditions than in the dark, due to both the opening of stomata and electron donor generation by photosynthesis.

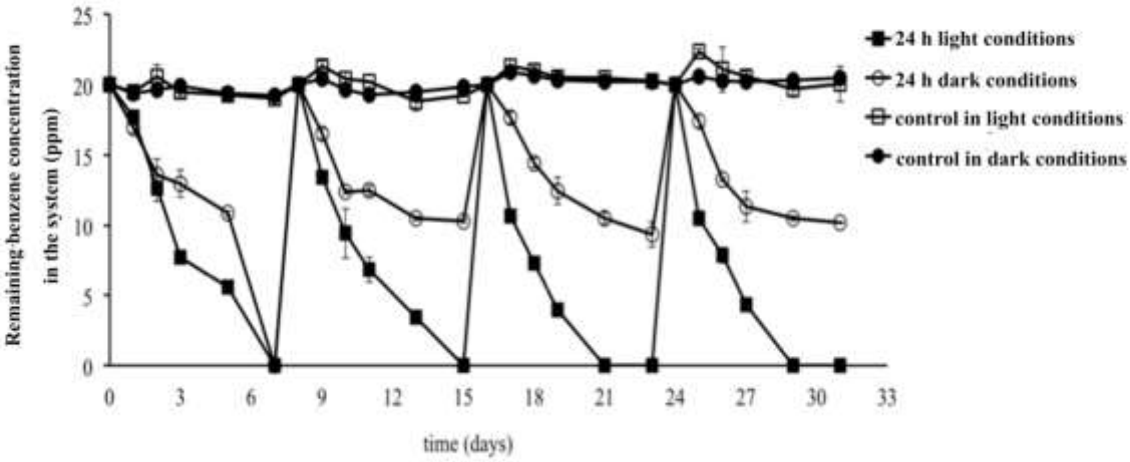


Figure 4.2 Remaining benzene concentration (ppm) in the system of 1st, 2nd, 3rd, and 4th cycle under 24 h light and 24 h dark conditions.

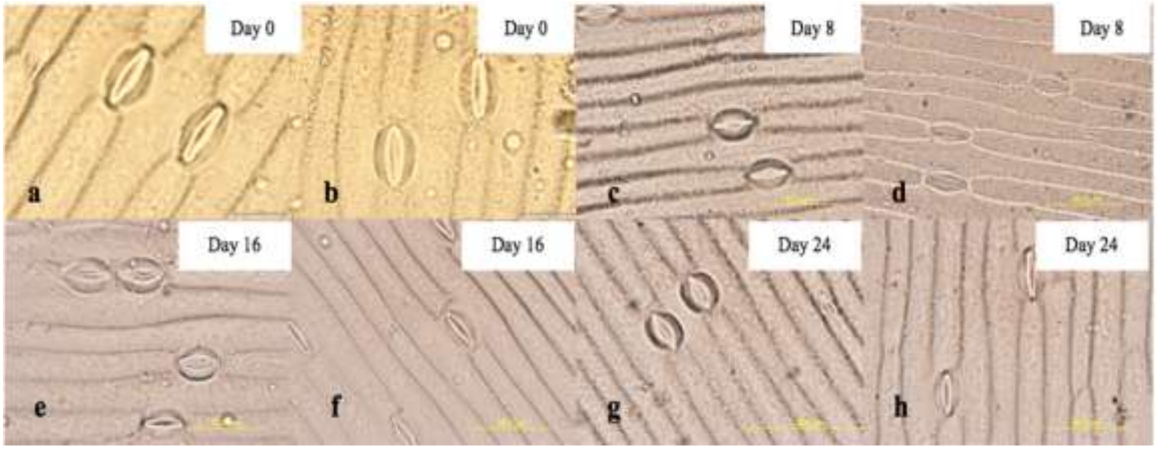


Figure 4.3 Stomata observation on the leaf of *D. sanderiana* under 24 h light conditions (a, c, e, g) and 24 h dark conditions (b, d, f, h) when exposure with 20 ppm of initial benzene concentration.

Benzene uptake by wax of *D. sanderiana*

Kvesitadze, *et al*. (2009) reported the possibility of benzene uptake by stomata and cuticle. The result suggested that although stomata had been known as a main pathway for benzene uptake by plant, some species can also take up high amounts of benzene through the cuticle. Not only research on environment, but also research on food, found the accumulation of benzene on crop leaf surfaces close to industrial locations (Gorna-Binkul, *et al*., 1996; Slaski, *et al*., 2000; Tsiros, *et al*., 1999; Collins, *et al*., 2000; Poborski, 1988; Reiderer, 1990; Kylin, *et al*., 1994). For example, in 1996, Gorna-Binkul, *et al.* (1996) reported benzene accumulation, including cucumber orange, parsley, cucumber (Gorna-Binkul, *et al*., 1996), apple and blackberry (Collins, *et al*., 2000). In theory, benzene that has 2.13 of log K_{ow} could transport easily through plant epidermises (Kamath, *et al*., 2004). The benzene-absorption efficiency of plant waxes was investigated to identify possible compounds for industrial application. In a preliminary experiment, crude wax of *D. sanderiana*, was extracted and used to adsorb benzene 72 h was required for the wax to reach saturation with benzene. The results showed that wax from 130 cm^2 of *D. sanderiana* leaf exposed to 20 ppm benzene, can uptake around 46 % of benzene, as shown in Fig 4.4. This result implied that the use of leaf material from some plant species for benzene adsorption is possible. This method has advantages of low cost and easy secondary disposal.

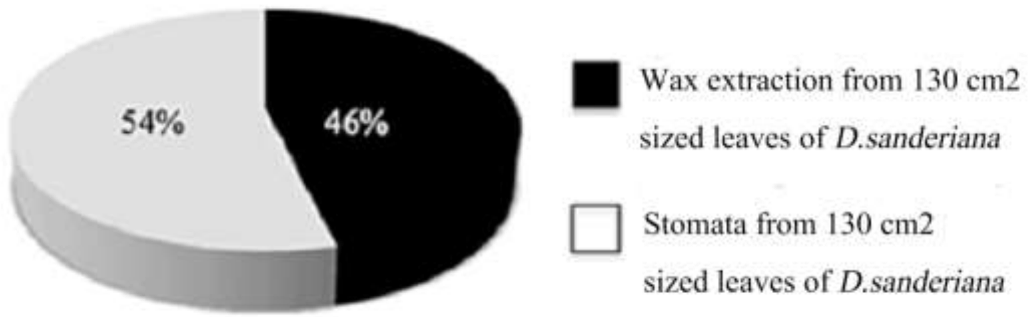


Figure 4.4 The ratios (%) of benzene uptake by wax and stomata of *D. sanderiana*, calculating base on 72 h exposure.

Benzene adsorption by biomaterials

Benzene adsorption by biomaterials in a static system

Since the cuticle of *D. sanderiana* adsorbed 46% of total benzene in a preliminary experiment, the leaves of other plant species might also adsorb high amounts of benzene, and for industrial application, the benzene adsorption by 21 plant materials was investigated. Benzene adsorbed per g of adsorbent was calculated and shown in Table 4.2. The dried leaf powders of *L. chinensis*, *D. picta, A. aureum, F. religiosa, L. macrocarpa, A. scholaris,* and *D. sanderiana* were the most efficient in benzene adsorption at day 3 of the experiment, although *L. chinensis* had relatively low benzene adsorption at day 1. Therefore, biomaterials from *D. picta*, *A. aureum*, *F. religiosa*, *L. macrocarpa*, *A. scholaris,* and *D. sanderiana* were suitable for application in a continuous system.

Table 4.2 Benzene adsorption efficiency by various dried leaf powders.

Plants Species	Benzene adsorption efficiency (µmole g^{-1} of adsorbent)		
	Day 1	**Day 2**	**Day 3**
Homalomena rubescens	0.92±0.01[a]	0.94±0.02[a]	1.10±0.28[a]
Citrus hystrix	7.64±0.33[d,e]	8.66±8.70[c,d,e,f]	9.19±2.63[b,c]
Musa paradisiaca	9.01±1.99[d,e,f]	15.20±3.29[g,h]	15.05±1.14[e]
Mangifera indica	7.67±3.45[d,e]	7.56±3.54[b,c,d,e]	6.79±3.05[b,c]
Catura metet	1.42±0.19[a]	3.75±0.52[a,b]	3.58±0.04[a]
Lagerstroemia inermis	3.80±0.16[b,c]	6.25±3.63[b,c,d]	9.45±0.88[c]
Cananga odorata	4.67±0.23[b,c]	5.59±1.32[b,c]	6.36±0.24[b]
Cassia siamea	3.52±0.34[b,c]	4.63±1.11[a,b,c]	7.66±0.17[b,c]
Bougain villea	3.38±0.37[b]	5.89±0.30[b,c,d]	7.06±1.17[b,c]
Litchi chinensis	3.29±0.59[b]	10.94±0.25[e,f,g]	23.46±2.10[h]
Coccinia grandis	4.62±1.84[b,c]	4.37±2.28[a,b,c]	6.64±0.33[b,c]
Dieffenbachia picta	10.73±0.25[f,g]	15.77±0.17[h]	19.37±0.25[f,g]
Attacus atlas	3.48±0.37[b,c]	17.49±0.46[h]	17.08±0.21[e,f]
Polyalthia longifolia	6.34±0.91[c,d]	7.98±0.44[b,c,d,e,f]	9.39±0.51[c]
Acrostichum aureum	10.97±0.63[g,h]	14.59±0.54[g,h]	18.90±0.54[f,g]
Ficus religiosa	10.61±0.12[f,g]	10.53±0.41[d,e,f,g]	18.33±1.12[f,g]
Lagerstroemia macrocarpa	10.94±0.54[f,g]	12.59±0.03[f,g,h]	20.07±0.88[h]
Alstonia scholaris	9.61±0.52[e,f]	14.25±0.38[g,h]	20.57±1.62[g,h]
Anthurium andraeanum	12.55±4.69[g,h]	12.56±0.21[f,g,h]	12.41±0.46[d]
Plerocarpus Indicus	13.88±2.52[h]	16.01±2.81[h]	17.82±4.35[f,g]
Dracaena sanderiana	11.00±4.68[f,g]	16.02±1.62[g,h]	19.00±2.8[f,g]

Data are list as average ± SD for three replications. Duncan's multiple range tests with 95% confident level was used to classify the group of data (a-h), symbol classify group of data following the column.

The relationship between benzene adsorption and quantity of wax

One factor that might affect benzene adsorption efficiency by dried leaf material is the quantity of wax. To investigate this, crude wax was extracted from the samples, and the relationship between quantity of wax and benzene removal efficiency was analyzed. The results demonstrate that in general material of higher wax content removes benzene to a greater amount than material of lower wax content. To confirm the relationship between quantity of wax and benzene adsorption by leaf material, the correlation coefficient was analyzed, and a high R-square value, equal to 0.6512 was found for the logarithmic curve (Fig 4.5). The result could be discussed in the term of thickness of wax because although high wax quantity materials could uptake benzene well, higher quantity of wax than 0.25 g g^{-1} absorbent, benzene had been uptake stably (Topp, *et al.*, 1986). Benzene transportation might be limited in how deep it can diffuse through the wax layer, so although high quantity of wax has higher thickness, benzene could not transport deeply to accumulate on the underside. In addition, some plant leaf material with low quantity of wax took up high benzene. In the other hand, some leaf materials have high wax quantity, but low benzene uptake had been found. These results suggest that not only quantity of wax but also wax composition might be a factor in benzene adsorption efficiency.

Table 4.3 Benzene adsorption efficiency by each plant leaf materials and their wax weight.

Plants species	Wax weight (mg g^{-1} of absorbent)	Benzene adsorption efficiency (μmole g^{-1} of adsorbent) at Day 3
Homalomena rubescens	0.01±0.00[a]	1.10±0.28[a]
Citrus hystrix	0.06±0.01[b,c]	9.19±2.63[b,c]
Musa paradisiaca	0.06±0.02[b,c]	15.05±1.14[e]
Mangifera indica	0.05±0.00[b,c]	6.79±3.05[b,c]
Catura metet	0.01±0.00[a]	3.58±0.04[a]
Lagerstroemia inermis	0.05±0.00[b,c]	9.45±0.88[c]
Cananga odorata	0.04±0.01[b]	6.36±0.24[b]
Cassia siamea	0.16±0.04[d]	7.66±0.17[b,c]
Bougain villea	0.05±0.00[b,c]	7.06±1.17[b,c]
Litchi chinensis	0.21±0.02[e]	23.46±2.10[h]
Coccinia grandis	0.05±0.00[b,c]	6.64±0.33[b,c]
Dieffenbachia picta	0.15±0.01[d]	19.37±0.25[f,g]
Attacus atlas	0.10±0.00[d]	17.08±0.21[e,f]
Polyalthia longifolia	0.07±0.01[c]	9.39±0.51[c]
Acrostichum aureum	0.10±0.00[d]	18.90±0.54[f,g]
Ficus religiosa	0.10±0.00[d]	18.33±1.12[f,g]
Lagerstroemia macrocarpa	0.18±0.03[e]	20.48±0.88[h]
Alstonia scholaris	0.11±0.01[d]	20.57±1.62[g,h]
Anthurium andraeanum	0.14±0.02[d]	12.41±0.46[d]
Plerocarpus Indicus	0.10±0.02[d]	17.82±4.35[f,g]
Dracaena sanderiana	0.25±0.00[e]	19.00±2.8[f,g]

Data are list as average ± SD for three replications. Duncan's multiple range tests with 95% confident level was used to classify the group of data (a-h), symbol classify group of data following the column.

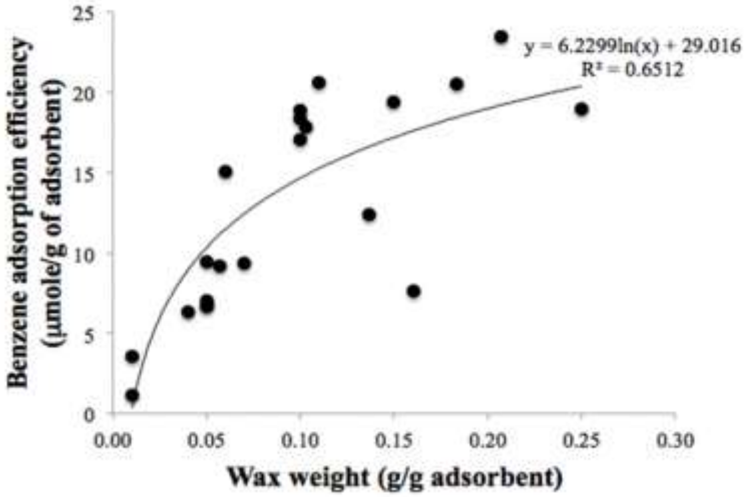


Figure 4.5 Relationship between benzene adsorption efficiency and wax quantity.

A comparison between benzene adsorption efficiency of various plant leaf materials grouped by wax weight is shown in Fig 4.6. The increasing of quantity of wax was considered as one factor affecting benzene adsorption, but there are still significant differences between plant materials with similar wax content. The result confirmed that the composition of wax could clearly be a factor affecting benzene adsorption by plant leaf materials.

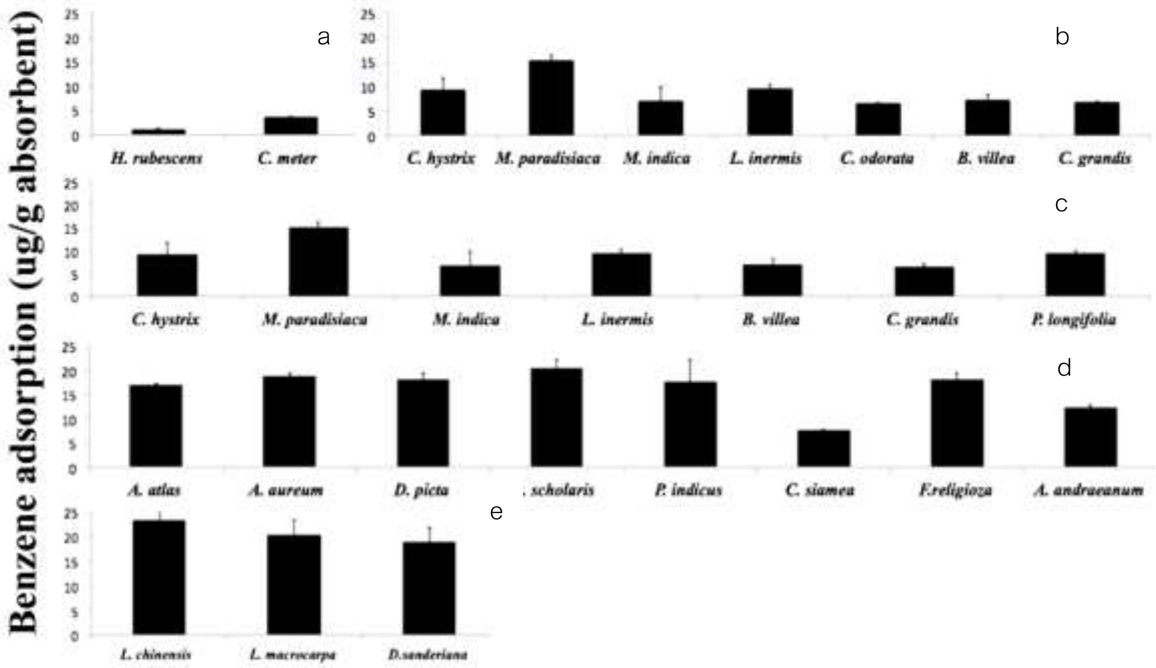


Figure 4.6 Comparison of benzene adsorption efficiency of plant leaf materials grouped by non-significantly difference in wax quantity by Duncan multiple range test: a) 0.01 - 0.03 mg g^{-1} of absorbent b) 0.04-0.06 mg g^{-1} of absorbent c) 0.05-0.07 mg g^{-1} of absorbent d) 0.1-0.16 mg g^{-1} of absorbent and e) 0.18-0.25 mg g^{-1} of absorbent (average and SD in each dot and error bar, respectively).

The relationship between benzene adsorption and composition of wax in each plant species

Fatty acids have been commonly known as a main composition of plant wax. The wax of selected plant leaf material was measured and analyzed by GC-MS (Fig 4.7). Material from *M. paradisiaca* leaves was chosen for analysis, as previous results showed that it has significantly higher benzene removal efficiency than other materials with similar wax content.

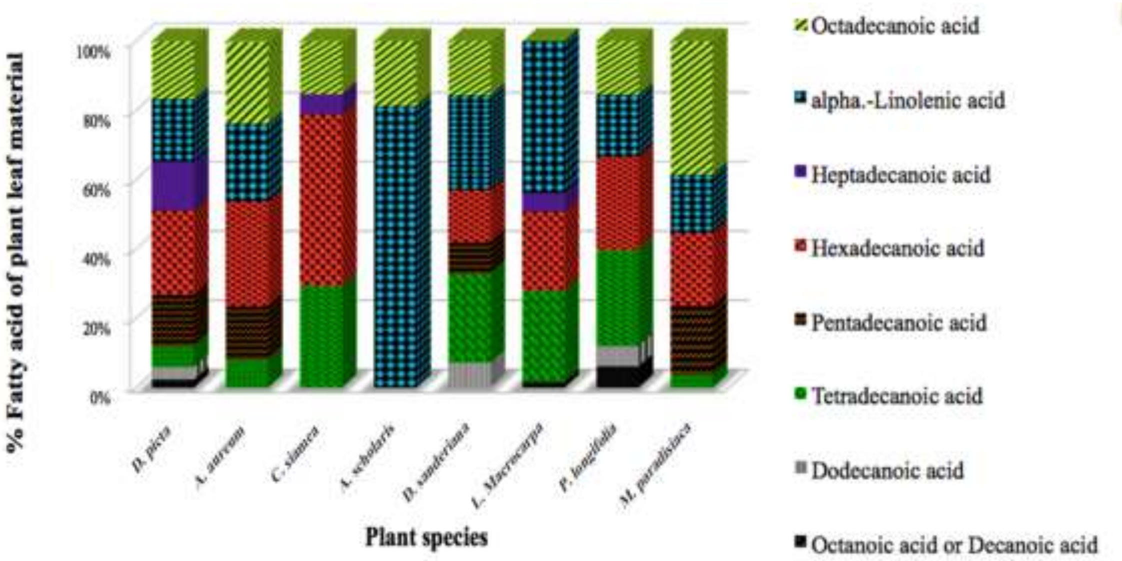


Figure 4.7 Percentage of fatty acid composition in wax of each plant leaf materials.

Plant leaf material from *P. longifolia* and *M. paradisiaca* are in the same grouping by wax weight, but *M. paradisiaca* leaf material was found to be more efficient in benzene removal (Fig 4.6). From the result, fatty acid composition was observed, and octadecanoic acid was found 38.5% of whole fatty acid composition in *M. paradisiaca* leaf material (Fig 4.7), and low among of octadecanoic acid had been found in *P. longifolia* leaf material.

Material from *C. siamea* was of interest because this material has significantly lower benzene removal efficiency (Fig 4.8b) compared with other materials with a similar wax weight. The fatty acid composition of *C. siamea* and three other plant materials of similar wax weight but higher benzene removal efficiency are shown in Fig 4.8a. The result suggested that alpha-linoleic acid may be the main factor for benzene removal, so material from *C. siamea*, which this fatty acid has not been found, have low benzene removal efficiency.

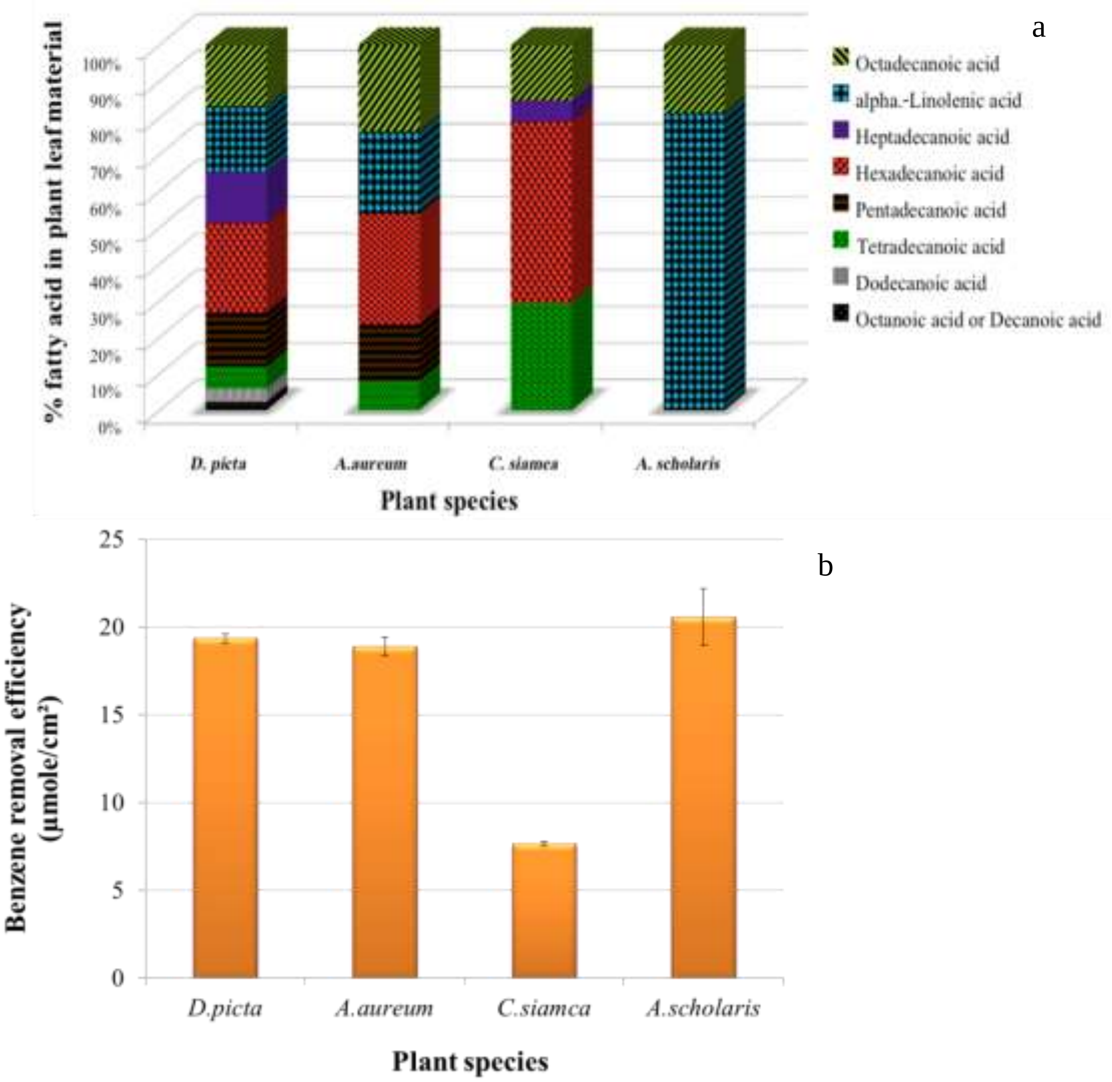


Figure 4.8 Percentage of fatty acid composition in wax of plant leaf materials that contain wax in the range of 0.10-0.16 mg g^{-1} of adsorbent (a) and benzene removal efficiency of these 4 plant leaf materials (b).

Alpha-linoleic acid was found in all species investigated except *C. siamea*. The increasing number of carbon on fatty acid molecules can decrease the polar fatty acids and might increase the solubility in benzene (Hoerr and Balston, 1944), so alpha-linoleic acid and octadecanoic acid might enhance benzene adsorption in plant leaf materials. A positive linear relationship between alpha-linolenic acid, but not octadecanoid acid, and benzene adsorption efficiency was identified (Fig 4.9).

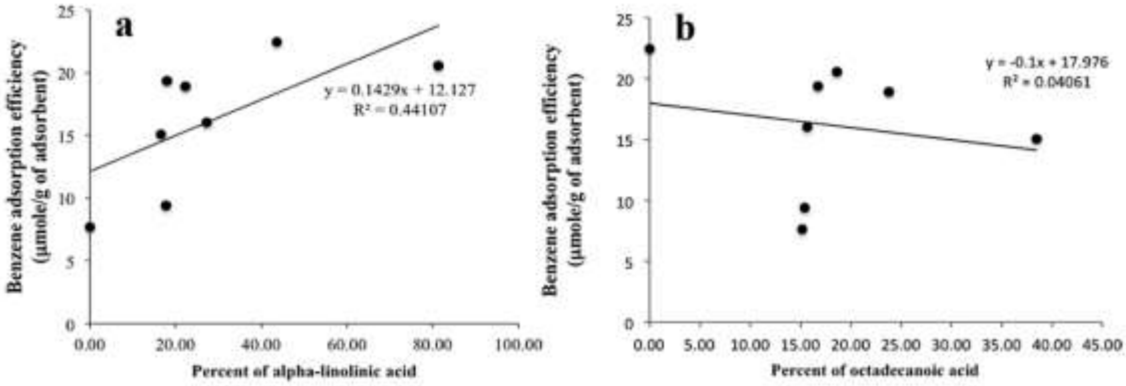


Figure 4.9 Linear regression of benzene adsorption efficiency and percentage of alpha-linoleic acid (a) and octadecanoic acid (b).

Fig 4.9 found that positive linear regression between benzene adsorption and percentage of alpha-linoleic acid had been present with 0.4410 of R-square, but linear regression between benzene adsorption and percentage of octadecanoic acid was not appeared. The result suggested that high quantity of alpha-linoleic acid might enhance benzene adsorption efficiency. Similar result had been found in xylene adsorption by pure alpha linoleic acid experiment. Not only fatty acid but also group of alkane was analyzed in Fig 4.10.

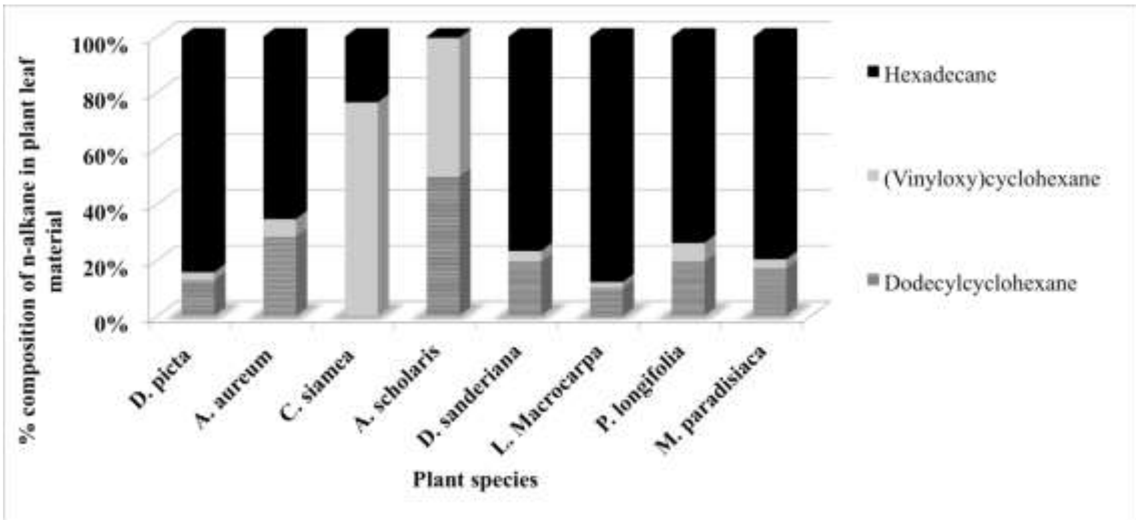


Figure 4.10 Percentage hexane composition in wax of each plant leaf materials.

High dodecyl cyclohexane in *A. scholaris* leaf material, which is a high benzene adsorption material, was found to be higher than other plant leaf materials. This result implied that dodecyl cyclohexane might also involve in benzene adsorption

Benzene adsorption by leaf material in a continuous system

For the application, the plant leaf materials of *D. picta, A. aureum, F.religiosa, L. macrocarpa, A. scholaris,* and *D.sanderiana* were ground and immobilized on glass beads. These materials were used as adsorbents in a continuous system with an air retention time of 3-5 min. Initial benzene of 55 ppm was controlled and continuous feed into the system. 80% benzene removal efficiency was found in *D. picta, A. aureum, A. scholaris,* and *D.sanderiana*, and 60% benzene removal efficiency was found in *F. religiosa, L. macrocarpa* (Fig 4.11). Most of plant materials had a limit capacity within 120 h. However *A. aureum* and *A. scholaris* leaf materials were occurred as the highest benzene removal capacity that had a limit capacity within 132 h.

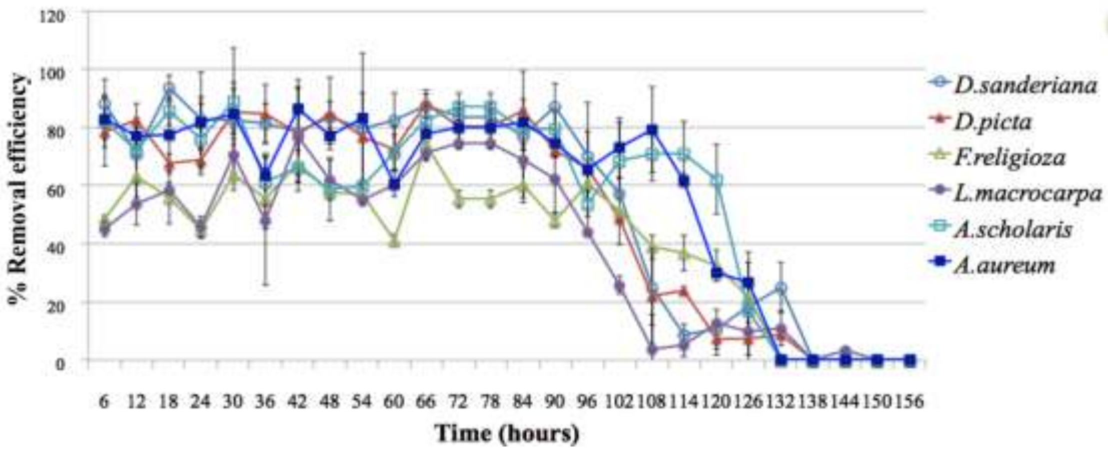


Figure 4.11 Benzene removal efficiency (%) in a continuous system of 6 plants biomaterials.

Benzene adsorption mechanisms

Capacity of each plant leaf material was calculated in the unit of umole/g absorbent and shown in Table 4.4. Benzene adsorption materials were desorbed by using hexane as an eluent. High quantity benzene desorption was found about 99% in selected materials.

Table 4.4 Benzene adsorption capacities of 6 plant biomaterials and percentage of benzene desorption in these selected materials.

	D.sanderiana	***D.picta***	***F.religioza***	***L.macrocarpa***	***A.aureum***	***A.scholaris***
Total porosity (cc g^{-1})	0.0703	0.0717	0.0461	0.1385	0.0694	0.2382
Micro pore volume (cc g^{-1})	0.0137	0.0181	0.0084	0.0176	0.0124	0.0377
Capacity (u mole/g)	20.37	20.32	16.08	20.77	21.15	21.50
%Desorption by hexane	>99.5%	>99.5%	>99.5%	>99.5%	>99.5%	83.11

Physical adsorption might be involved for benzene adsorption mechanism in wax of these materials because hexane can desorb benzene easily from most of plant leaf materials. However only 83.11% benzene desorption was occurred in *A.scholaris* leaf material because high porosity was found clearly in biomaterial from this plant leaf. Physical sorption mechanism was confirmed by FT-IR result. Functional groups on adsorbent surface were observed (Fig 4.12). In addition, although a few studies reported benzene adsorption mechanism by plant leaf materials or other natural materials, benzene adsorption by activated carbon presented that physical adsorption is the main benzene adsorption mechanism (Ramirez, *et al.*, 2005; Rozwadowski and Wojsz, 1987; Stoeckli, *et al*., 2001)

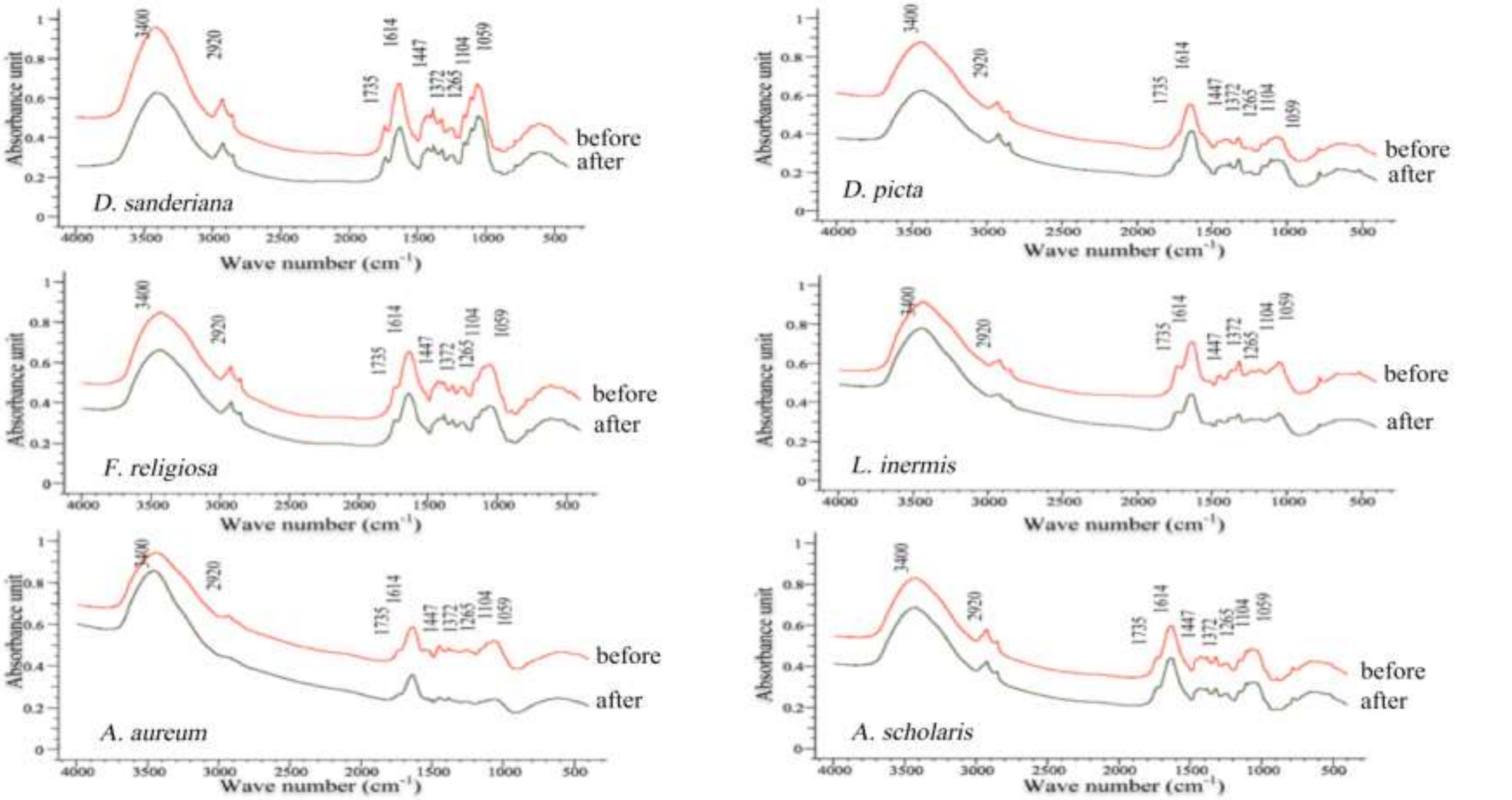


Figure 4.12 Functional groups on the surface of adsorbent before and after benzene adsorption in selected plant leaf material.

Fig 4.12 shows the same tendency of functional groups of plant leaf material surface before and after benzene treatment process, and the same pattern of functional group of every plant leaf material surfaces were found as (O-H, around 3400 cm^{-1} and C-H, at 2920 cm^{-1}). In addition, many fingerprint regions were found between 1800 and 1000 cm^{-1}. The peaks that were observed at 1,735 and 1,614 cm^{-1} showed C=O and C=C, respectively. A Methoxy (O-CH_3) group was also found at 1,447 cm^{-1}. At 1,372 and 1,265 cm^{-1}, a fingerprint region was presented as O-H deformation originating from the phenolic group, and C-OH in primary alcohol and secondary alcohol was shown around 1,059 cm^{-1} and 1,104 cm^{-1}, respectively. This result suggested that the main mechanism for benzene adsorption by plant leaf materials should be physical adsorption.

Chapter 5 Conclusions

D. sanderiana has the highest benzene removal efficiency of 8 ornamental plants that were screened for benzene removal at an initial benzene concentration of 20 ppm for 72 h under room temperature in a static system. The plant was exposed to 20 ppm of initial benzene concentration for 4 cycles: each cycle refers to 72 h fumigation under room temperature. The result suggests that benzene removal by *D. sanderiana* can be a sustainable technology because this plant can still uptake high benzene even though it was exposed for a long period of time. In addition, benzene uptake in the 2^{nd}, 3^{rd}, 4^{th} cycles were found to be faster than in the first cycle because the plant needed time to adapt when it was under stress conditions. Dark and light conditions can affect benzene uptake by *D. sanderiana*. Under light conditions, *D. sanderiana* showed higher benzene removal than under dark conditions. Stomata, which are normally reported as a main benzene uptake pathway, were observed under light and dark conditions. The result showed that stomata were closed under dark conditions. The result suggests that the closing of stomata under dark conditions can decrease benzene removal efficiency of *D. sanderiana*. However, the cuticle of the plant cans still uptake benzene. Benzene uptake under light and dark conditions with benzene uptake pathways of stomata and cuticle was calculated to be 54% and 46%, respectively. The cuticle of *D. sanderiana* also took up high levels of benzene. The application of plant wax to adsorb benzene was also studied. Plant leaves from 21 species such as *Homalomena rubescens, Citrus hystrix, Musa paradisiaca, Mangifera indica, Catura metet, Lagerstroemia inermis, Cananga odorata, Cassia siamea, Bougain villea, Litchi chinensis, Coccinia grandis, Dieffenbachia picta, Attacus atlas, Polyalthia longifolia, Acrostichum aureum, Ficus religiosa, Alstonia scholaris, Anthurium andraeanum, Plerocarpus Indicus, Lagerstroemia macrocarpa,* and *Dracaena sanderiana* were used to adsorb 20 ppm of benzene in a static system at room temperature for 72 h. Materials from 6 species of plant leaf such as *Dieffenbachia picta, Acrostichum aureum, Ficus religiosa, Lagerstroemia macrocarpa, Alstonia scholaris,* and *Dracaena sanderiana* were used to adsorb benzene. Wax from selected plant leaf materials were extracted and analyzed both for their quantity of wax and wax composition. The relationship of benzene adsorption efficiency and quantity of wax was found to be a logarithmic curve with R^2=0.65. The result suggests that increasing wax quantity can enhance benzene adsorption efficiency. Wax composition analysis showed that plants with high benzene adsorption efficiency contain high quantities of alpha-linoleic acid and dodecyl cyclohexane. A linear regression of the relationship between benzene adsorption efficiency and quantity of alpha-linoleic showed R^2=0.44. Alpha-linoleic acid can be a key factor for benzene adsorption. For industry application, plant leaf materials from 6 plant species were immobilized on glass beads by cassava glue and were applied in a continuous adsorption system. The plant leaf materials with the highest benzene adsorption capacity were obtained from *A. aureum* and *A. scholaris*. The study of benzene adsorption mechanism by plant leaf materials involved physical adsorption because benzene was easily desorbed by hexane. The adsorption mechanism was confirmed by FT-IR. Similar function groups on the surface of the materials before and after benzene adsorption were not different.

References

Aloko, D. F. and Adebayo, G. A., 2007, "Production and Characterization of Activated Carbon from Agricultural Waste (Rice-husk and corn-cob)", **Journal of Engineering and Applied Sciences**, Vol. 2, pp. 440-444.

Atkinson, T., 2007, "A review of the Role of Benzene Metabolites and Mechanisms in Malignant Transformation: Summative Evidence for a Lack of Research in Non-myelogenous Cancer Types", **International Journal of Hygiene and Environmental Health**, Vol. 212, pp. 1–10.

Auria, R., Morales, M., Acuna, M. E., Perez, F., and Revah, S., 1996, "Biofiltration of Toluene Vapors: Startup and Gaseous Ammonia Addition", **In: ProcConf Biofiltration: an Air Pollution Control Technology**, USC, LA, pp. 134–141.

Bagne´ris C., Cammack, R., and Mason, J. R., 2004, "Subtle Difference between Benzene and Toluene Dioxygenases of *Pseudomonas putida"*, **Applied and Envionmental Microbiology**, pp 1570–1580.

Barona, A., Elias, A., Arias, R., Cano, I., and Gonzalez, R., 2004, "Biofilter Response to Gradual and Sudden Variations in Operating Conditions". **Biochemical Engineering Journal**, Vol. 22, pp. 25–31.

Beck, K. R. and Moore, L. G., 1997, "Extraction of Cotton Impurities: Supercritical CO_2 vs. SoxhIeVTCE". **Textile Chemist and Colorist**, Vol. 29, pp. 88-70.

Bohn, H. L., 1992, "Considering Biofiltration for Decontaminating Gases", **Chemical engineering progress**, Vol. 88, pp. 34–40.

Bouchez, M., Blanchet, D., and Vandecasteele, J. P., 1995, "Degradation of Polycyclic Aromatic Hydrocarbons by Pure Strains and by Defined Strain Associations: Inhibition Phenomena and Cometabolism", **Applied Microbiology and Biotechnology**, Vol. 43, pp. 156-164.

Caron Link, **Properties of Activated Carbon** [Online]. Available: http://www.activated-carbon.com/1-3.html (10-01-2011).

Chablain, P. A., Zgoda, A. L., Sarde, C. O., and Truffaut, N., 2001, "Genetic and Molecular Organization of the Alkylbenzene Catabolism Operon in the Psychrotrophic Strain *Pseudomonas putida* 01G3", **Applied and Envionmental Microbiology**, Vol. 67, pp. 453.

Canadian Environmental Protection Act, 1999, **Part VII Controlling Pollution and Managing Wastes: Division 4 (Fuels)**, pp. 33.

Choi E. N., Cho, M. C., Kim, Y., Kim, C., and Lee, K., 2003, "Expansion of Browth Substrate Range in *Pseudomonas putida* F1 by Mutations in Both cymR and todS, Which Recruit a Ring-fission Hydrolase CmtE and Induce the tod Catabolic Operon, respectively", **Microbiology**, Vol. 149, pp. 795–805.

Clark, O. G., Edeogu, I., Feddes, J., Coleman, R. N., and Abolghasemi. A., 2004, "Effects of Operating Temperature and Supplemental Nutrients in a Pilot• Scale Agricultural Biofilter", **Canadian Biosystems Engineering**, Vol. 46, pp. 6.7-6.16.

Costura, R. K. and Alvarez, P. J. J., 2000, "Expression and Longevity of Toluene Dioxygenase in *Pseudomonas putida* F1 Induced at Different Dissolved Oxygen Concentrations", **Water Research**, Vol. 34, pp. 3014-3018.

Devinny, J. S., Deshusses, M. A., and Webster, T. S., 1999, **Biofiltration for Air Pollution Control**, Lewis, Boca Raton.

Edwards, E. A. and Grbic´-Galic´, D., 1992, "Complete Mineralization of Benzene by Aquifer Microorganisms under Strictly Anaerobic Conditions". **Applied Environmental Microbiology**, Vol. 58, pp. 2663-2666.

Edwards, E. A., Wills, L. E., Reinhard, M., and Grbic-Galic, D., 1992, "Anaerobic Degradationof Toluene and Xylene by Aquifer Microorganisms under Sulfate-Reducing Conditions". **Applied Environmental Microbiology**, Vol. 58, pp. 794-800.

Edwards, E. A. and Gribic-Galic, D., 1994, "Anaerobic Degradation of Toluene and o-Xylene by a Methanogenic Consortium". **Applied Environmental Microbiology**, Vol. 60, pp. 313–322.

Collins, C. D., Bell, J. N. B., and Crews, C., 2000, "Benzene Accumulation in Horticultural Crops". **Chemosphere**, Vol. 40, pp. 109-114.

Devinny, J. S., Deshusses, M. A., and Webster, T. S., 1999, "Biofiltration for Air Pollution Control". **CRC press. California**, pp. 6-258.

Gibson, D. T., Koch, J. R., and Kallio, R. E., 1968, "Oxidative Degradation of Aromatic Hydrocarbons by Microorganisms. I. Enzymatic Formation of Catechol from Benzene". **Biochemistry**, Vol. **7**, pp. 2653–2662.

Gibson, D. T. and Parales, R. E., 2000, "Aromatic Hydrocarbon Dioxygenases in Environmental Biotechnology", **Current Opinion in Biotechnology**, Vol. 11, pp. 236–243.

Gorna-Binkul, A., Keymeulen, R., Van Langenhove, and H., Buszewski, B., 1996, "Determination of Monocyclic Aromatic Hydrocarbons in Fruit and Vegetables by Gas Chromatography-mass Spectrometry". **Journal of Chromatography A**, Vol. 734, pp. 297-302.

Harwood, C. S., 1989, "A Methyl-Accepting Protein is Involved in Benzoate Taxis in *Pseudomonas putida"*. **Journal of Bacteriology**, Vol. 171, pp. 4603-4608.

Harwood, C. S., Parales, R. E., Dispensa, M., 1990, "Chemotaxis of *Pseudomonas putida* Toward Chlorinated Benzoates". **Applied Environmental Microbiology**, Vol. 56, pp. 1501–1503.

Heald, S. C. and Jenkins, R. O., 1996, "Expression and Substrate Specificity of the Toluene Dioxygenase of *Pseudomonas putida* NCIMB 11767". **Applied Microbiology and Biotechnology**, Vol. 45, pp. 56–62.

Hodge, D. S., Medina, V. F., Islander, R. L., and Devinny, J. S., 1991, "Treatment of Hydrocarbon Fuel Vapors in Biofilters". **Environmental Technology**, Vol. 12, pp. 655–662.

Hoerr, C. W. and Ralstok, W., 1944, "The Solubilities of the Normal Saturated Fatty Acids", ***the Journal of Organic Chemistry***, Vol. *09*, pp. 329–337.

Juteau, P., Beaudet, R., McSween, G., Lépine, F., and Bisaillon, J. G., 1996, "Study of the Reductive Dechlorination of Pentachlorophenol by a Methanogenic Consortium", **Canadian Journal of Microbiology**, Vol. 41, pp. 862-868.

Kamath, R., Rentz, J. A., Schnoor, J. L., and Alvarez, P. J. J., 2004," Phytoremediation of Hydrocarbon-Contaminated Soils: Principles and Applications", **Vazquez-Duhalt, R. and Quintero-Ramirez, R., eds. Studies in Surface Science and Catalysis**. Elsevier, Amsterdam, pp. 447-478.

Kennesá, C., Wuá, W. M., Bhatnagar, L., and Zeikus, J. G., 1996, "Anaerobic Dechlorination and Mineralization of Pentachlorophenol and 2,4,6-Trichlorophenol by Methanogenic Pentachlorophenol Degrading Granules", **Applied Microbiology and Biotechnology**, Vol. 44, pp. 801-806.

Kermani, K., Pourmoghaddas, H., Bina, B., and Khazaei, Z., 2006, "Removal of Phenol from Aqueous Solutions by Rice Husk Ash and Activated Carbon", **Pakistan Journal of Biological Sciences**, Vol. 9, pp. 1905-1910.

Kinney, K. A., Chang, D. P. Y., Schroeder, E. D., and Scow, K. M., 1996. "Performance of a Directionally-Switching Biofilter Treating Toluene Contaminated Air", **Proc 89th Annu Meet Exhibition Air and Waste Management Association**, Nashville.

Knoll, G. and Winter, J., 1987. "Anaerobic Degradation of Phenol in Sewage Sludge: Benzoate Formation from Phenol and Carbon Dioxide in the Presence of Hydrogen", **Applied Microbiology and Biotechnology**, Vol. 25, pp. 384–391.

Knoll, G. and Winter, J., 1989. "Degradation of Phenol via Carboxylation to Benzoate by a Defined, Obligate Syntrophic Consortium of Anaerobic Bacteria", **Applied and Environmental Microbiology**, Vol. 30, pp. 318–324.

Kobayashi, T., Hashinaga, T., Mikami, E., and Suzuki, T., 1989. "Methanogenic Degradation of Phenol and Benzoate in Acclimated Sludges". **Water science and technology**, Vol. 21, pp. 55–65.

Kragelund, L. and Nybroe, O., 1994. "Culturability and Expression of Outer Membrane Proteins during Carbon, Nitrogen, or Phosphorus Starvation of *Pseudomonas fluorescens* DF57 and *Pseudomonas putida* DF14", **Applied and Environmental Microbiology**, Vol. 60, pp. 2944-2948.

Kvesitadze, G., and Kvesitadze, E., 2009. "Potential of Higher Plants as Environmental Remediators", **Bulletin of the Georgian National Academy of Sciences**, Vol. 175.

Kvesitadze, E., Sadunishvili, T., and Kvesitadze, G., 2009, "Mechanisms of organic Contaminants Uptake and Degradation in Plants", **Engineering and technology**, Vol. 55, pp. 458-468.

Kylin, H., Grimvall, E., and Ostman, C., 1994. "Environmental Monitoring of Polychlorinated Biphenyls using Pine Needles as Passive Samples", **Environmental Science and Technology**, Vol. 28, pp. 1320-1324.

Leson, G. and winter A. M., 1991, "Biofiltration: an Innovative Air Pollution Control Technology for VOC Emissions", **Journal of the Air and Waste Management Association**, Vol. 41, pp. 1045–1054.

Liu, Y., Mu, Y., Zhu, Y., Ding, H., and Arens, N., 2007. "Which Ornamental Plant Species Effectively Remove Benzene from Indoor Air?", **Atmospheric Environment**, Vol. 41, pp. 650–654.

Marsh, A., 1994, "Biofiltration for Emission Abatement", **European Coatings Journal**, Vol. 7, pp. 528–536.

McMurry J., 2003, "Fundamentals of Organic Chemistry (Fifth ed.)", Agnus McDonald, 409, ISBN 0-534-39573-2.

Michel P., **Adsorption** [Online], http://perso.limsi.fr/mpons/indexENG.html, 09/2008.

Morgenroth, E., Schoreder, E. D., Chang D. P. Y., and Scow, K. M., 1996, "Nutrient Limitation in a Compost Biofilter Degradation Hexane", **Journal of the Air and Waste Management Association**, Vol. 46, pp. 300-308.

Morsen, A. and Rehm, H. J., 1987, "Degradation of Phenol by a Mixed Culture of *Pseudomonas putida* and *Cryptococcus elinovi* Adsorbed on Activated Carbon", **Applied Microbiology and Biotechnology**, Vol. 26, pp. 283-288.

Orwell, R. L., Wood, R., Tarran, J., Torpy, F., and Burchett, M., 2004. **Removal of Benzene by the Indoor Plants/ Substrate Microcosm and Implications for Air Quality. Plants and Environmental Quality Group**, Faculty of Science, University of Technology, Sydney, Westbourne St, Gore Hill, NSW 2065, Australia.

Ottengraf, S. P. P., Meesters, J. J. P., Van Den Oever, A. H. C., and Rozema, H. R., 1986, "Biological Elimination of Volatile Xenobiotic Compounds Using Biofilters", **Bioprogress Eng**., Vol. 1, pp. 61.

Parales, R. E., Ditty, J. L., and Harwood, C. S., 2000, "Toluene -Degrading Bacteria are Chemotactic towards the Environmental Pollutants Benzene, Toluene, and Trichloroethylene", **Applied and Environmental Microbiology**, Vol. 66, pp. 4098.

Poborski, P., 1988, "Pollutant Penetration through the Cuticle". In " Air Pollution and Plant Metabolism", S. Schulte-Hostede et al. (Eds.), **Elsevier**, London, UK, 19-35.

Pollution Control Department, 2007, **"Development of Environmental and Emission Standards of Volatile Organic Compounds (VOCs) in Thailand"**, Bangkok, Thailand.

Rahul, Mathur, A. K., and Balomajumder, C., 2013, "Biological Treatment and Modeling Aspect of BTEX Abatement Process in a Biofilter", **Bioresource Technology**, Vol. 142, pp. 9–17.

Ramírez, C., Adelung, R., Kunz, R., Kipp, L., and Schattke, W., 2005, "Lithium Adsorption by TiSe2 of Varying Concentration via Density Functional Theory", **Physical Review B**, Vol. 71, pp. 035426.

Rappaport, S. M., Kim, S., Thomas, R., Johnson, B. A., Bois, F. Y., and Kupper, L. L., 2013, "Low-Dose Metabolism of Benzene in Humans: Science and Obfuscation", **Carcinogenesis**, Vol. 34, pp. 2-9.

Reardon, K. F., Mosteller, D. C., and Bull Rogers, J. D., 2000, "Biodegradation Kinetics of Benzene, Toluene, and Phenol as Single and Mixed Substrates for *Pseudomonas putida* F1", **Biotechnology and bioengineering**, Vol. 69, pp. 385-400.

Ribera, R. G., 2001, “Production of Poly-hydroxyal kanoates by *Pseudomonas putida* KT2442 Harbouring pSK2665 in Wastewater from Olive Oil Mills (alpechín)”, **Electronic Journal of Biotechnology**, Vol. 4.

Riederer, M., 1990, Estimating Partitioning and Transport of Organic Chemicals in the Foliage Atmosphere System-Discussion of a Fugacity-Based Model. **Environmental Science and Technology**, Vol. 24, pp. 829-937.

Rozwadowski, M. and Wojsz R., 1987, Microporous Adsorbent Sieve Effect on Thermodynamic Functions for State of Perturbation in Adsorbed Molecules, **Journal of Inclusion Phenomena**, Vol. 5, pp. 297-305.

Sapref, (ND). **Oil Refining**, http://www.sapref.com/about/WhatWeDo.

Sato, M., 1966, “Metabolism of Phenolic Substances by the Chloroplasts. II. Conversion by the Isolated Chloroplasts of p-Coumaric Acid to Caffeic Acid”**, Phytochemistry**, Vol. 5, pp. 385–389.

Schnatter, A., Rosamili, K., and Wojcik, N., 2005, “Review of the Literature on Benzene Exposure and Leukemia Subtypes”, **Chemico-Biological Interactions**, Vol. 153-154, pp. 9-21.

Sciencelab, 2001, **Benzene**. http://www.sciencelab.com/msds.php?msdsId=9927339.

Sechneska, M., Tomova, N., and Dechev, G., 1968,“Ascorbate Oxidase Activity of Spinach Chloroplasts”, **Comptes Rendus de Academie Bulgare des Sciences**, Vol. 21, pp. 277-280.

Shields, M. S., Montgomery, S. O., Chapman, P. J., Cuskey, S. M., and Pritchard, P. H., 1989,“Novel Pathway of Toluene Catabolism in Trichloroethylene-Degrading Bacterium G4”, **Applied and Environmental Microbiology**, Vol. 55, pp. 1624-1629.

Shimko, I. G., Spasov, V. A., Chinennya, S. K., Zakirova, R. I., Tananina, I. N., Perchugor, G. Y., and Pavlova, O. I., 1988, “Biochemical Method Soffreeing Gas-Air Mixture from Sulphur Containing Compounds”, **Fibre Chemistry**, Vol. 19, pp. 373-378.

Singh, O. V. and Jain, R. K., 2003, “Phytoremediation of Toxic Aromatic Pollutants from Soil”, **Applied Microbiology and Biotechnology**, Vol. 63, pp. 128–135.

Slaski, J. J., Archambault, D. J., and Li, X., 2000, **The Potential Use of PAH Accumulation as a Marker of Exposure to Air Emission from Oil and Gas Flares**, Air Research Users Group, Alberta Environment, Edmonton, Alberta.

Sriprapat, W., and Thiravetyan, P., 2013, “Phytoremediation of BTEX from Indoor Air by *Zamioculacas zamiifolia”*, **Water Air Soil Pollution**, Vol. 224, pp. 1482.

Stoeckli, F., Moreno, C., Carrasco, F., and López, V. M., 2001, "Distribution of Oxygen Complexes on Activated Carbons from Immersion Calorimetry, Titration and Temperature-Programmed Desorption Techniques", **Carbon**, Vol. 39, pp. 2235-2237.

Swift, R. J., Carter, S. E., Widdowson, D. A., Mason, J. R., and Leak, D. J., 2001, "Expression of Benzene Dioxygenase from *Pseudomonas putida* ML2 in is-1,2-Cyclohexanediol-Degrading *Pseudomonads*", **Applied Microbiology and Biotechnology**, Vol. 55, pp. 721-726.

Tarran, J., Torpy, F., and Burchett, M., 2007, **Use of Living Pot-Plants to Cleanse Indoor Air-Review**, Faculty of Science, University of Technology Sydney (UTS) PO Box 123, Broadway, NSW 2007, Sydney, Australia.

Topp, E., Scheunert, I., Attar, A., and Korte, F., 1986, "Factors Affecting the Uptake of C-14-Labelled Organic Chemicals by Plants from Soil", **Ecotoxicology and Environmental Safety**, Vol. 11, pp. 219–228.

Tsiros, I. X., and Ambrose, R. B., Chronopoulou-sereli, A., 1999, "Air Vegetation Soil Partition of Toxic Chemicals in Environmental Simulation Modeling", **GlobalNEST International Journal**, Vol. 1, pp. 177-184.

Ugrekhelidze, D., Korte, F., and Kvesitadz, G., 1997, "Uptake and Transformation of Benzene and Toluene by Plant Leaves". **Ecotoxicology and environmental Safety**, vol. 37, pp. 24-29.

U.S. Environmental Protection Agency, 2007, **EPA Region 6: Human Health Medium-Specific Screening Levels**. Texas, USA.

U.S. Environmental Protection Agency, 200, **Benzene**, http://www.epa.gov/teach/ [Online] 2/27/2009.

Van Lith, C., David, S. L., and Marsh, R., 1990, "Design Criteria for Bio filters". In: VanLith, C., David, S. L., Marsh, R. (eds) "Effluent Treatment and Waste Disposal". Clair Tech, Utrecht, **Netherlands Institution of Chemical Engineers SympSer**, Vol. 116, pp. 127–132.

Weckhuysen, B., Vriens, L., and Verachtert, H., 1993, "The Effect of Nutrient Supple Mentation on the Biofiltration Removal of Butanolin Contaminated Air", **Applied Microbiology and Biotechnology**, Vol. 39, pp. 395–399.

Weckhuysen, B., Vriens, L., and Verachtert, H., 1994, "Biotreatment of Ammonia and Butanal Containing Waste Gases", **Applied Microbiology and Biotechnology**, Vol. 42, pp. 147–152.

Wever, H. D., Cort, S. D., Noots, I., and Verachtert, H., 1997, "Isolation and Characterization of *Rhodococcus rhodochrous* for The Degradation of the Wastewater Component 2-hydroxybenzothiazole", **Applied Microbiology and Biotechnology**, Vol. 47, pp. 458-461.

Whited, G. M. and Gibson, D. T., 1991, "Toluene-4-Monooxygenase, a Three Component Enzyme System That Catalyzes the Oxidation of Toluene to p-Cresol in *Pseudomonas mendocina* KR1", **Journal of Bacteriology**, pp. 3010-3016.

Wolverton, B. C., 1996, **How to grow fresh air. penguin book**, New York.

Wolverton, B.C., Johnson, A., and Bounds, K., 1989, **Interior Landscape Plants for Indoor Air Pollution Abatement, Final Report**, September N.A.S.A., Stennis Space Centre MS.

Wood, R., Orwell, R., Tarran, J., and Burchett, M., 2001, **Pot-Plants Really Do Clean Indoor Air**. The Nursery Papers, 2001/2, NIAA (Nursery Ind. Assocn. Aust.) Sydney, Australia.

Wu, W-M., Bhatnagar, L., and Zeikus, J. G., 1993, Performance of Anaerobic Granules for Degradation of Pentachlorophenol, **Applied and Environmental Microbiology**, Vol. 59, pp. 389-397.

Yu, H., Kim, B. J., and Rittmann, B. E., 2001, "The Roles of Intermediates in Biodegradation of Benzene, Toluene, and p-Xylene by *Pseudomonas putida* F1", **Biodegradation**, Vol. 12, pp. 455–463.

Zarook, S., 2005, **Biotechnology for odor and air pollution control**, Germany, pp. 29-168.