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Combined photocatalysis and phytoremediation for efficient treatment of polybromodiphenyl ethers (PBDES)

Ka Lai Chow

Hong Kong Baptist University

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COMBINED PHOTOCATALYSIS
AND PHYTOREMIEDIATION FOR
EFFICIENT TREATMENT OF
POLYBROMODIPHENYL ETHERS
(PBDES)

CHOW KA LAI
Ph. D. Thesis

HONG KONG BAPTIST UNIVERSITY
2013
DECLARATION

I hereby declare that this thesis represents my own work which has been done after registration for the degree of PhD at Hong Kong Baptist University, and has not been previously included in a thesis or dissertation submitted to this or any other institution for a degree, diploma or other qualifications.

Signature: __________________

Date: November 2013
ABSTRACT

Brominated flame retardants have been widely used in industry. There is a rapidly growing public concern about their ubiquity in the environment. This project investigated the possible removal treatments of polybrominated diphenyl ethers (PBDEs) using two treatment methods: (I) photocatalysis by TiO$_2$ and (II) phytoremediation by *Oryza sativa* and *Phragmites australis*, and a combined system involving these two technologies. Advanced oxidation process (AOP) is a promising technology for removing emerging chemicals. In this case, nano-scaled titanium (IV) oxide was applied to evaluate its capability in the degradation of BDE-209 under visible light. The residual PBDE congeners after reaction were analyzed by gas chromatography-mass spectrometry (GC-MS). The half-life for removing BDE-209 by TiO$_2$ was 3.05 days under visible light. Tetra- and penta-BDEs were the major degraded products of BDE-209. Optimum conditions for photocatalytical degradation of BDE-209 was found to be pH 12 (93% ± 1%), at least 5 mg/L (93 ± 1.70%) of humic acid and in the form of anatase/rutile TiO$_2$ (82% ± 3%). Incomplete removal of PBDEs by water treatment plants and point-source contamination may lead to their discharge into water bodies and ultimately into soils. Consequently, the second part of the project was phytoremediation of PBDEs. Uptake of BDE-209 by rice cultivars, namely Fengmeizhan, Hefengzhan and Guangyinzhan, and common reed were examined by 60-day cultivation in sterilized BDE-209 spiked soil. Hefengzhan possessed the greatest ability in the removal and accumulation of BDE-209 among the three cultivars, especially when associated with *Glomus intraradices*, at pH 7. A series of plant-contamination sorption analyses were also employed for pathway studies of PBDEs uptake by rice. A partition-limited model was applied for describing and estimating the uptake of BDE-209 by rice roots. The average quasi-equilibrium factors ($\alpha_{pt}$) of BDE-47, -99 and -209 in root uptake were $1.44 \times 10^{-3}$, $0.966 \times 10^{-3}$ and $0.115 \times 10^{-3}$ in sand ($< 1$), implying a non-equilibrium state of the movement of molecules and a dominant passive transport uptake. From the result of sorption analysis of dead and fresh roots, the apoplastic pathway likely dominated the transport of PBDEs into root cells. These results provide essential information on the uptake mechanism of PBDEs into plants. Based on the results from photocatalysis and phytoremediation, a combined photocatalysis (TiO$_2$ and visible light) and constructed wetland system (*Oryza sativa* (Hefengzhan) and *Phragmites australis* (common reed) was set up for comparing PBDEs removal efficiencies. The removal percentages of BDE-209 in the combined system were
found to be promoted when compared to the individual systems, which could be explained by enhanced biodegradability of PBDEs in photocatalysis. Therefore, the proposed advanced wastewater treatment technology (combined photocatalysis and constructed wetland systems) might help to degrade and eliminate BDE-209 in the wastewater, and thus reduce the risks of marine contamination by discharging these incompletely or partially treated wastewaters containing PBDEs.
ACKNOWLEDGEMENTS

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<tbody>
<tr>
<td>AM</td>
<td>Arbuscular mycorrhizal</td>
</tr>
<tr>
<td>AOP</td>
<td>Advanced oxidation processes</td>
</tr>
<tr>
<td>BDE-47</td>
<td>2,2',4,4'-tetrabromodiphenyl ether</td>
</tr>
<tr>
<td>BDE-99</td>
<td>2,2',4,4',5-pentabromodiphenyl ether</td>
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<tr>
<td>BDE-100</td>
<td>2,2',4,4',6-pentabromodiphenyl ether</td>
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<tr>
<td>BDE-153</td>
<td>2,2',4,4',5,5'-hexabromodiphenyl ether</td>
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<tr>
<td>BDE-154</td>
<td>2,2',4,4',5,6'-tetabromo-diphenyl ether</td>
</tr>
<tr>
<td>BDE-209</td>
<td>Decabromodiphenyl ether</td>
</tr>
<tr>
<td>BFR</td>
<td>Brominated flame retardant</td>
</tr>
<tr>
<td>BGC</td>
<td>Bank of Glomales in China</td>
</tr>
<tr>
<td>Ctrl</td>
<td>Non-spiking control</td>
</tr>
<tr>
<td>CB</td>
<td>Conduction band</td>
</tr>
<tr>
<td>CEPT</td>
<td>Chemically enhanced primary treatment</td>
</tr>
<tr>
<td>DBP</td>
<td>Disinfection by-product</td>
</tr>
<tr>
<td>DCF</td>
<td>2',7'-dichlorofluorescin diacetate</td>
</tr>
<tr>
<td>DCM</td>
<td>Dichloromethane</td>
</tr>
<tr>
<td>DDT</td>
<td>Dichlorodiphenyltrichloroethane</td>
</tr>
<tr>
<td>D.I.</td>
<td>Deionized Water</td>
</tr>
<tr>
<td>DMSO</td>
<td>Dimethyl sulfoxide</td>
</tr>
<tr>
<td>DNB</td>
<td>Dinitrobenzene</td>
</tr>
<tr>
<td>DNT</td>
<td>Dinitrotoluene</td>
</tr>
<tr>
<td>DOC</td>
<td>Dissolved organic carbon</td>
</tr>
<tr>
<td>EDCs</td>
<td>Endocrine disrupting compounds</td>
</tr>
<tr>
<td>E-waste</td>
<td>Electronic waste</td>
</tr>
<tr>
<td>EU</td>
<td>European Union</td>
</tr>
<tr>
<td>FSH</td>
<td>Follicle stimulating hormone</td>
</tr>
<tr>
<td>GC-MS</td>
<td>Gas chromatography-mass spectrometry</td>
</tr>
<tr>
<td>GI</td>
<td>Glomus intraradices</td>
</tr>
<tr>
<td>GM</td>
<td>Glomus mosseae</td>
</tr>
<tr>
<td>GV</td>
<td>Glomus versiforme</td>
</tr>
<tr>
<td>GST</td>
<td>Glutathione-transferase</td>
</tr>
<tr>
<td>H2DCF-DA</td>
<td>Dichlorofluorescein diacetate</td>
</tr>
<tr>
<td>H2O2</td>
<td>Hydrogen peroxide</td>
</tr>
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<td>HA</td>
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<td>HCl</td>
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</tr>
<tr>
<td>Hi</td>
<td>Henry’s law constant</td>
</tr>
<tr>
<td>HRT</td>
<td>Hydraulic residence time</td>
</tr>
<tr>
<td>HSC</td>
<td>Hematopoietic stem cells</td>
</tr>
<tr>
<td>KOH</td>
<td>Potassium hydroxide</td>
</tr>
<tr>
<td>LIN</td>
<td>Lindan</td>
</tr>
<tr>
<td>LH</td>
<td>Luteinizing hormone</td>
</tr>
</tbody>
</table>
LTP  Lipid transfer protein
LOD  Limit of detection
MassDEP  Massachusetts Department of Environmental Protection
MBDE-MXE  Mass-labelled \(^{13}\)C\(_{12}\) solution/mixture
NAMF  Control with no AMF inoculation
NaOH  Sodium hydroxide
NaR  Nitroreductase
NOEL  No-observed-effect-level
n-ZVI  Nanoscale zerovalent iron
OH\(^{-}\)  Hydroxide
OsPT  *Oryza sativa* phosphate transporter
PAH  Polycyclic aromatic hydrocarbons
PBDEs  Polybrominated diphenyl ethers
PBS  Phosphate-buffered solution
PCBs  Polychlorinated biphenyls
PHN  Phenanthrene
PM\(_{2.5}\)  Particulate matter 2.5 micrometers
PRD  Pearl River Delta
PUR  Polyurethane
PYR  Pyrene
RFU  Relative fluorescence units
ROS  Reactive oxygen species
T4  Thyroxin
TCB  1,2,3-trichlorobenzene
TiO\(_{2}\)  Titanium dioxide
TOC  Total organic carbon
TPEM  Two-photon excitation microscopy
TRE  Total removal efficiency
TSP  Total suspended particles
TSH  Thyroid-stimulating hormone
SCISTW  Stonecutters Island sewage treatment work
SDS-DBP  Simulated distribution system disinfection by-product
SHBG  Sex hormone binding globulin
SOM  Soil organic matter
SQ  Squarylium cyanine dye
STSTW  Sha Tin sewage treatment work
UV  Ultraviolet
WWTP  Wastewater treatment plants
\(\alpha_{pt}\)  Quasi-equilibrium factor
\(f_{pom}\)  Fraction of the organic matter in the plant
\(f_{pw}\)  Fraction of the water in the plant
<table>
<thead>
<tr>
<th>Symbol</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>$^{13}$C$_{12}$-PBDEs</td>
<td>Mass-labelled polybrominated diphenyl ethers</td>
</tr>
<tr>
<td>$C_s$</td>
<td>Contaminant in the soil</td>
</tr>
<tr>
<td>$C_{stem/C_{root}}$</td>
<td>Translocation factor</td>
</tr>
<tr>
<td>$C_{som}$</td>
<td>SOM-normalized contaminant concentration in soil</td>
</tr>
<tr>
<td>$K_d$</td>
<td>Partition coefficients</td>
</tr>
<tr>
<td>$K_{ow}$</td>
<td>Partition coefficients</td>
</tr>
<tr>
<td>$K_{pom}$</td>
<td>Partition coefficient between plant organic matter and plant water</td>
</tr>
<tr>
<td>$K_{som}$</td>
<td>Partition coefficient between SOM and water,</td>
</tr>
<tr>
<td>4NP</td>
<td>4-nitrophenol</td>
</tr>
</tbody>
</table>
CHAPTER 1
GENERAL INTRODUCTION

1.1 Research Background

The rapid development of materials and technologies, accompanied with new compounds, pharmaceuticals and chemicals has generated public concern on their potential threats to the environmental and human beings. Brominated flame retardants are widely used in plastics, textiles and furnishing foam to diminish their flammability by hindering the combustion of the polymeric materials. Polybromodiphenyl ethers or polybrominated diphenyl ethers (PBDEs) are brominated flame retardants, which are distributed worldwide in the environment, including air, sediment and soil (Deng et al., 2007; Wang et al., 2005). Illegal and inappropriate dumping and recycling of electronic waste (e-waste) containing PBDEs could be one of the major reasons for the leakage of high concentration of PBDEs into the environment (Leung et al., 2007). Current wastewater treatment plants may not be able to eliminate PBDEs. For instance, 2,2',4,4'-tetrabromodiphenyl ether (BDE-47), 2,2',4,4',5-pentabromodiphenyl ether (BDE-99) and decabromodiphenyl ether (BDE-209) could still be identified in sludge and effluents discharged from a tertiary sewage treatment facility in California (North, 2004). Owing to their high persistence and lipophilicity,
PBDEs have raised public concern. Reports have shown bioaccumulation of PBDEs in animals, such as birds, seals and also human beings including blood, breast milk and adipose tissue (Meerts, 2001). Exposure to these chemicals is suspected to cause estrogen disruption, hypothyroidism and abnormal development in neural system (Meerts, et. al., 2001; Costa et al., 2008; Eriksson et al., 2002). Consequently, incomplete removal of these contaminants by water treatment plants and point-source contamination may lead to discharge into water bodies and adversely affect living organisms (Bolong et al., 2009).

1.2 Emerging Contaminants

The Massachusetts Department of Environmental Protection (MassDEP) has defined emerging contaminants as chemicals or materials of interest with the following characterizations (MassDEP, 2006):

- a known or real threat to human health or environment.
- without a published health standard or a developing standard.
- insufficient or limited toxicological information or toxicity information which is available for re-evaluation or
- a contaminant may be "emerging" due to the discovery of a new source, a new pathway to humans, or a new detection method or technology.
This group of contaminants causes negative effects not necessarily due to their persistence but continuous introduction into the environment. Therefore, their high transformation and removal rates can be overwhelmed by their consistent extraordinarily influx. This group of contaminants can be found in daily used products, such as pharmaceuticals, hormones, personal care products and some industrial additives (Petrovic’ et al., 2003).

1.3 Polybrominated Diphenyl Ethers (PBDEs) Characteristics and Their Applications

Since the 1970s, the demand for brominated flame retardants has been increasing (Wang et al., 2007). The annual global production of polybrominated diphenyl ethers (PBDEs) increased from 40,000 tonnes (Arias, 1992), to around 67,000 tonnes between 1992 to 2001 (BSEF, 2010). Asian countries shared about 40% of the global demand for PBDEs (BSEF, 2010). PBDEs are an important class of brominated flame retardants with a high production rate. They are generally used in polymer and textile products as additive flame retardants (de Wit, 2002; Rahman et al., 2001). PBDEs added resins or polymers are also common components of electrical appliances, contributing to the release of PBDEs from electronic-waste (e-waste) (WHO, 1994). The chemical structure of PBDEs are similar to those of polychlorinated biphenyls (PCBs) and
dichlorodiphenyltrichloroethane (DDT), and therefore their persistence and environmental distribution are similar (Rahman et al., 2001). PBDEs contain a large group of congeners which vary in the number and positions of the bromine atoms on the two phenyl rings (Fig. 1.1). There are 209 possible congeners of PBDEs in total, which are grouped into mono-, di-, tri-, tetra-, penta-, hexa-, hepta-, octa-, nona- and deca-bromodiphenyl ethers respectively according to the number of bromide ions they bear. There are 3, 12, 24, 42, 46, 42, 24, 12, 3 and 1 isomers for mono-, di-, tri-, tetra-, penta-, hexa-, hepta-, octa-, nona- and deca-bromodiphenyl ethers respectively (WHO, 1994). The BDE congener numbers and the chemical composition of some commonly studied PBDEs are listed in Table 1.1 and 1.2.

During heating, a brominated flame retardant is able to generate bromine-free radicals which will eliminate other free radicals in the flame propagation process, thereby resisting the flame from spreading (Richardson, 2008). Most commercial brominated flame retardants are made of mixtures of PBDEs, their isomers and homologues. The common commercial brominated flame retardant mixture can be classified into three groups, namely commercial deca-BDE, octa-BDE and penta-BDE, each containing various compositions of brominated diphenyl ethers (ATSDR, 2004a). Although the European Union (EU)
Figure 1.1 The chemical structure of PBDEs

\[
\text{Br}_x \quad \text{O} \quad \text{Br}_y
\]
Table 1.1 BDE congener numbers and chemical composition of the commonly studied congeners (US EPA, 2010).

<table>
<thead>
<tr>
<th>Homologs of PBDEs</th>
<th>IUPAC number</th>
<th>Chemical formula</th>
</tr>
</thead>
<tbody>
<tr>
<td>MonoBDE</td>
<td>BDE 3</td>
<td>4-BDE</td>
</tr>
<tr>
<td>DiBDE</td>
<td>BDE 7</td>
<td>2,4-BDE</td>
</tr>
<tr>
<td>DiBDE</td>
<td>BDE 15</td>
<td>4,4'-BDE</td>
</tr>
<tr>
<td>TriBDE</td>
<td>BDE 17</td>
<td>2,2',4-BDE</td>
</tr>
<tr>
<td>TriBDE</td>
<td>BDE 28</td>
<td>2,4,4'-BDE</td>
</tr>
<tr>
<td>TetraBDE</td>
<td>BDE 47</td>
<td>2,2',4,4'-BDE</td>
</tr>
<tr>
<td>TetraBDE</td>
<td>BDE 66</td>
<td>2,3',4,4'-BDE</td>
</tr>
<tr>
<td>TetraBDE</td>
<td>BDE 49</td>
<td>2,2',4,5'-BDE</td>
</tr>
<tr>
<td>TetraBDE</td>
<td>BDE 71</td>
<td>2,3',4,6-BDE</td>
</tr>
<tr>
<td>PentaBDE</td>
<td>BDE 85</td>
<td>2,2',3,4,4'-BDE</td>
</tr>
<tr>
<td>PentaBDE</td>
<td>BDE 99</td>
<td>2,2',4,4',5-BDE</td>
</tr>
<tr>
<td>PentaBDE</td>
<td>BDE 100</td>
<td>2,2',4,4',6-BDE</td>
</tr>
<tr>
<td>PentaBDE</td>
<td>BDE 119</td>
<td>2,3',4,4',6-BDE</td>
</tr>
<tr>
<td>PentaBDE</td>
<td>BDE 126</td>
<td>3,3',4,4',5-BDE</td>
</tr>
<tr>
<td>PentaBDE</td>
<td>BDE 138</td>
<td>2,2',3,4,4',5'-BDE</td>
</tr>
<tr>
<td>HexaBDE</td>
<td>BDE 153</td>
<td>2,2',4,4',5,5'-BDE</td>
</tr>
<tr>
<td>HexaBDE</td>
<td>BDE 154</td>
<td>2,2',4,4',5,6'-BDE</td>
</tr>
<tr>
<td>HexaBDE</td>
<td>BDE 156</td>
<td>2,3,3',4,4',5-BDE</td>
</tr>
<tr>
<td>HeptaBDE</td>
<td>BDE 183</td>
<td>2,2',3,4,4',5,6-BDE</td>
</tr>
<tr>
<td>HeptaBDE</td>
<td>BDE 184</td>
<td>2,2',3,4,4',6,6'-BDE</td>
</tr>
<tr>
<td>HeptaBDE</td>
<td>BDE 191</td>
<td>2,3,3',4,4',5,6-BDE</td>
</tr>
<tr>
<td>OctaBDE</td>
<td>BDE 196</td>
<td>2,2',3,3',4,4',5,6-BDE</td>
</tr>
<tr>
<td>OctaBDE</td>
<td>BDE 197</td>
<td>2,2',3,3',4,4',6,6'-BDE</td>
</tr>
<tr>
<td>NonaBDE</td>
<td>BDE 206</td>
<td>2,2',3,3',4,4',5,5,6-BDE</td>
</tr>
<tr>
<td>NonaBDE</td>
<td>BDE 207</td>
<td>2,2',3,3',4,4',5,6,6'-BDE</td>
</tr>
<tr>
<td>DecaBDE</td>
<td>BDE 209</td>
<td>2,2',3,3',4,4',5,5,6,6'-BDE</td>
</tr>
</tbody>
</table>
Table 1.2 Chemical and physical information on PBDEs.

<table>
<thead>
<tr>
<th>PBDE congeners</th>
<th>Chemical formula</th>
<th>Molecular weight (g)</th>
<th>Water solubility at 25°C (μg/L)</th>
<th>Henry’s law constant (Hi) (Pa m^3 mol^-1) at 25°C</th>
<th>Log $K_{ow}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>BDE-47</td>
<td>$C_{12}H_5Br_6O$</td>
<td>485.8</td>
<td>11</td>
<td>0.85</td>
<td>6.81</td>
</tr>
<tr>
<td>BDE-99</td>
<td>$C_{12}H_5Br_5O$</td>
<td>564.7</td>
<td>2.4</td>
<td>0.6</td>
<td>6.5–8.4</td>
</tr>
<tr>
<td>BDE-209</td>
<td>$C_{12}Br_{10}O$</td>
<td>959.17</td>
<td>&lt;0.1</td>
<td>0.04</td>
<td>6.3–12.6</td>
</tr>
</tbody>
</table>

^aUS EPA, 2008a, ^bUS EPA, 2008b, ^cUS EPA, 2008c
banned penta-BDEs in 2004, the penta-BDEs can still be released from degradation, recycling and disposal of electronic products (Cox and Ethymiou, 2003; Hale et al., 2002; Alcock et al., 2003). Among these commercial PBDE products, deca-BDEs occupy the majority of production and consumption (30000 tonnes /year), which is about 75% (Sjödin et al., 1999). They are mainly applied in electronic enclosures, such as television cabinets (ATSDR, 2004a). The majority of deca-BDE from Asia is produced in eastern China, such as Laizhou Bay, Shandong Province (Wang et al., 2007, Jin et al., 2009). The release of PBDEs into the environment can be attributed to the synthesis process, inappropriate disposal, incorporation into products, and from hazardous waste sites (DEPA, 1999).

1.4 Sources of PBDEs in the Environment

Illegal and inappropriate dumping and recycling of e-waste are believed to be the two major sources of PBDEs contamination in the environment (Wang et al., 2005). Large amounts of PBDEs were discharged into the environment during manufacture and disposal of consumer products containing PBDEs. PBDEs may be present in soil, air and sediments. For example, PBDEs (2720–4250 ng/g, dry w) were found in soils from an acid-leaching site (using acids to recover metals from electronic boards) of Guiyu (Leung et al., 2007). Twenty-two PBDE
congeners (BDE-3, -7, -15, -17, -28, -49, -71, -47, -66, -77, -100, -119, -99, -85, -126, -154, -153, -138, -156, -184, -183, -191) in total suspended particles (TSP) and particulate matter 2.5 micrometers (PM$_{2.5}$) particles at Guiyu were detected (Deng et al., 2007). The monthly concentrations of the sum of 22 BDE congeners in the TSP and PM$_{2.5}$ samples were 21.5 and 16.6 ng m$^{-3}$, respectively, with the majority contributed by nine congeners (BDE-28, -47, -66, -100, -99, -154, -153, -183 and -191) (74.5 and 84.3%, respectively) (Deng et al., 2007). Unintentional ingestion of dust can be a significant source of PBDEs exposure to human beings, especially to small children (Frederiksen et al., 2009). BDE-209 was the most abundant BDE congener found in home dust around the Pearl River Delta, followed by BDE-99 and BDE-47. For children, the daily intake of PBDEs through non-dietary ingestion of dust (101-404 ng/day) was higher than that through food consumption (77-190 ng/day) (Kang et al., 2011). A total of 43 PBDE congeners (concentrations varied from 0.80 to 824 ng/g (dry weight)) were detected in soil and river sediment samples collected near an open e-waste treatment site in Guiyu, Guangdong, China (Wang et al., 2005). Evidence for bioaccumulation of PBDEs was found in the aquatic food web, involving bivalves and fish (Yu et al., 2010; Luo et al., 2007). Due to this bioaccumulative potential, exposure to PBDEs in the human diet is mainly contributed by consumption of
fish (Frederiksen et al., 2009), and accumulation in human blood and breast milk (Bi et al., 2006). A significant positive relationship was found between total PBDE concentrations in human milk and the frequency of dietary intake of fish and shellfish (Ohta et al., 2002). Biota and human samples were predominated by five PBDE congeners (BDE-47, -99, -100, -153, -154) (Costa et al., 2008).

1.5 PBDEs in the Human Body and Their Adverse Health Effects

Endocrine disrupting compounds (EDCs) are man-made and naturally occurring chemicals that are able to mimic hormones and thus capable of disrupting normal endocrine system (Richardson, 2008). According to the types of targets that they exert effect, EDCs can be classified into three categories: estrogenic (compounds that imitate or block natural estrogens), androgenic (compounds that imitate or block natural testosterone), and thyroidal (compounds with direct and/or indirect impacts on the thyroid) (Gültekin and Ince, 2007). PBDEs were reported to be thyroidal EDCs and disrupt the thyroid hormone system in three ways: (a) function and regulation of thyroid gland; (b) metabolism of thyroid hormone; and (c) transportation of thyroid hormone (Brouwer et al., 1998). In addition, PBDEs were also estrogenic that it may bind to and activate estrogen receptor (Meerts et al., 2001).

1.5.1 Toxicity of BDE-47
The presence of BDE-47 as the recalcitrant residue in the environment would probably be the result of the degradation of higher congener compounds (Rahman et. al., 2001). It was the dominant congener detected in the breast milk samples from the patients in the Guangzhou Second People’s Hospital (Bi et al., 2006). BDE-47 has been reported to exhibit anti-androgenic characteristics by inhibiting the binding of androgens to the androgen receptor in vitro based on an animal study involving rodents (Stoker et al., 2005). In addition, BDE-47 disrupted thyroid hormone activity in mice after a 14-day exposure period (Hallgren et al., 2001), leading to histologic and morphometric changes of thyroid glands (Talsness et al., 2008). Reproductive toxicity was also observed in rodent experiments of BDE-47 exposure. The ovarian weight, tertiary follicles and serum estradiol concentration were decreased after the administration of BDE-47 (Talsness et al., 2008). In a study of the mechanisms of BDE 47-mediated injury to primary human fetal liver hematopoietic stem cells (HSCs), BDE-47 was found to induce oxidative stress and the onset of apoptosis in primary human fetal liver HSCs and thus might influence the hematopoiesis during fetal development (Shao et al., 2008). Exposure to PBDEs might lead to lower thyroid-stimulating hormone (TSH) during pregnancy, which may eventually affect the normal development of fetal brain (Chevrier et al., 2010).
1.5.2 Toxicity of BDE-99

Exposure to BDE-99 posed persistent disturbances in spontaneous motor behavior and dysfunction in learning and memory in adult mice and rats (Eriksson et al., 2002; Branchi et al., 2003), attributed to the free radicals production (Cheng et al., 2009). BDE-99 can exert toxic reproductive effects to both male and female rats. It was found that sperm count was reduced and the ultrastructure of ovary cells was changed after In utero exposure to low dose of BDE-99 (Kuriyama et al., 2005; Talsness et al., 2005). Furthermore, BDE-99 may exert endocrine disrupting effects, such as reducing thyroxin (T4) concentration in female rats (Kuriyama et al., 2007). It has been noted that BDE-99 concentrations in dust was negatively correlated with luteinizing hormone (LH) and follicle stimulating hormone (FSH), but positively correlated with inhibin B and sex hormone binding globulin (SHBG) (Meeker et al., 2009).

1.5.3 Toxicity of BDE-209

BDE-209 is the major component of the commercial deca-BDE flame retardant. It has been revealed that the soils in Guiyu, China are polluted by PBDEs with BDE-209 the most dominant congener which occupied 35 to 82% of the total PBDEs content (Leung et al., 2007). It has been reported that adult mice suffered from neurobehavioral derangements after the administration of BDE-209
during a definite period of neonatal brain development (Viberg et al., 2003). Another study also showed that neonatal exposure to BDE-209 led to dose-response changes in spontaneous behavior and cholinergic susceptibility in adult mice (Johansson et al., 2008). Generally, BDE-209 is less toxic to rats than the other lower brominated congeners, e.g. NOEL (no-observed-effect-level) values in subchronic toxicity studies of rats are usually in less than 10 mg/kg/day for penta-BDE, but much more for deca-BDE (g/kg/day range). (Costa and Giordano, 2007). However, degradation of BDE-209 probably leads to prominent production of BDE-47 and -99, and therefore their toxicities would be of concern (Sun et. al., 2009).

1.6 PBDEs in Sewage

A 200% increase in domestic production of commercial deca-BDE mixture was observed in China (from 10,000 to about 30,000 tonnes) between 2000 and 2005 due to the rapid growth of manufacturing industry in electronic products (Chen et al., 2007). However, most existing wastewater treatment facilities are not specifically designed for eliminating emerging chemicals, including PBDEs. Table 1.3 compares PBDE concentrations in wastewaters and sewage sludge from China with those of other countries. Generally, most of the PBDEs are deposited in sludge after sewage treatment (Table 1.3). For instance,
Table 1.3 Influent (ng/L), effluent (ng/L), sludge (ng/g dw.) and total removal efficiency (TRE) (%) for BDE-47, -99, -209 and total PBDEs in Stonecutters Island sewage treatment work (SCISTW) and Sha Tin sewage treatment work (STSTW), and compared with other countries

<table>
<thead>
<tr>
<th>Location</th>
<th>Sewage sample</th>
<th>BDE-47</th>
<th>BDE-99</th>
<th>BDE-209</th>
<th>Total PBDEs</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hong Kong, China</td>
<td>Influent</td>
<td>7.09 ± 2.30</td>
<td>3.03 ± 1.02</td>
<td>46.7 ± 19.2</td>
<td>66.1 ± 22.5</td>
<td>Man et al., 2013</td>
</tr>
<tr>
<td>SCISTW</td>
<td>Effluent</td>
<td>2.01 ± 1.34</td>
<td>0.47 ± 0.464</td>
<td>1.51 ± 0.506</td>
<td>7.17 ± 2.14</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Sludge</td>
<td>13.9 ± 6.01</td>
<td>9.16 ± 0.661</td>
<td>47.4 ± 11.9</td>
<td>103 ± 46.8</td>
<td></td>
</tr>
<tr>
<td></td>
<td>TRE</td>
<td>71.6 ± 15.8 (%)</td>
<td>84.7 ± 12.3 (%)</td>
<td>96.0 ± 2.62 (%)</td>
<td>87.4 ± 8.02 (%)</td>
<td></td>
</tr>
<tr>
<td>Hong Kong, China</td>
<td>Influent</td>
<td>5.10 ± 1.25</td>
<td>2.71 ± 0.565</td>
<td>14.5 ± 6.1</td>
<td>30.4 ± 8.63</td>
<td></td>
</tr>
<tr>
<td>STSTW</td>
<td>Effluent</td>
<td>1.12 ± 0.724</td>
<td>0.283 ± 0.344</td>
<td>0.5 ± 0.2</td>
<td>3.3 ± 1.41</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Sludge</td>
<td>29.7 ± 7.59</td>
<td>14.7 ± 2.56</td>
<td>87.9 ± 31.7</td>
<td>162 ± 52.9</td>
<td></td>
</tr>
<tr>
<td></td>
<td>TRE</td>
<td>74.8 ± 19.5 (%)</td>
<td>90.7 ± 9.14 (%)</td>
<td>96.2 ± 2.41 (%)</td>
<td>89.3 ± 2.62 (%)</td>
<td></td>
</tr>
<tr>
<td>Canada</td>
<td>Influent</td>
<td>102 ± 83</td>
<td>121 ± 93</td>
<td>169</td>
<td>265 ± 210</td>
<td>Rayne and Ikonomou,</td>
</tr>
<tr>
<td></td>
<td>Effluent</td>
<td>14 ± 4</td>
<td>16 ± 4</td>
<td>9</td>
<td>36 ± 10</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Sludge</td>
<td>963 ± 415</td>
<td>1247 ± 516</td>
<td>746</td>
<td>2698 ± 1141</td>
<td></td>
</tr>
<tr>
<td></td>
<td>TRE</td>
<td>82.3 (%)</td>
<td>86.8 (%)</td>
<td>94.7 (%)</td>
<td>86.4 (%)</td>
<td></td>
</tr>
<tr>
<td>Guangzhou, China</td>
<td>Influent</td>
<td>3.48 (0.500 - 11.1)</td>
<td>3.41 (0.349 - 10.488)</td>
<td>550 (11.6 - 2413)</td>
<td>568 (14.0 - 2467)</td>
<td>Peng et al., 2009</td>
</tr>
<tr>
<td></td>
<td>Effluent</td>
<td>0.123 (0.083 - 0.228)</td>
<td>0.098 (0.059 - 0.205)</td>
<td>2.6 (0.6 - 3.4)</td>
<td>3.25 (1.01 - 4.52)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Sludge</td>
<td>14 (3.2 - 26.7)</td>
<td>17.7 (3.1 - 35.4)</td>
<td>6586 (150 - 22894)</td>
<td>6665 (165 - 23052)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>TRE</td>
<td>96.5 (%)</td>
<td>97.1 (%)</td>
<td>99.5 (%)</td>
<td>99.4 (%)</td>
<td></td>
</tr>
<tr>
<td>California, USA</td>
<td>Sludge</td>
<td>757 ± 31</td>
<td>944 ± 43</td>
<td>1183 ± 227</td>
<td>3381 ± 335</td>
<td>North, 2004</td>
</tr>
<tr>
<td></td>
<td>Effluent</td>
<td>10.47 ± 0.212</td>
<td>11.2 ± 0.2</td>
<td>1.73 ± 0.652</td>
<td>29.0 ± 1.49</td>
<td></td>
</tr>
<tr>
<td>Germany</td>
<td>Sludge</td>
<td>47.9 (&lt;8 - 115)</td>
<td>53.9 (8.7 - 124)</td>
<td>429 (97.1 - 2 217)</td>
<td>555 (142 - 2 491)</td>
<td>Knoth et al., 2007</td>
</tr>
<tr>
<td>Australia</td>
<td>Sludge</td>
<td>140 ± 110</td>
<td>140 ± 120</td>
<td>720 ± 980</td>
<td>1137 ± 1136</td>
<td>Clarke et al., 2008</td>
</tr>
<tr>
<td>Kuwait</td>
<td>Sludge</td>
<td>2.33 (0.2 - 7.8)</td>
<td>4.72 (0.4 - 14.7)</td>
<td>182 (4.8 - 1596)</td>
<td>191 (5.7 - 1560)</td>
<td>Gevao et al., 2008</td>
</tr>
<tr>
<td>Switzerland</td>
<td>Sludge</td>
<td>44.4 (19.8 - 128)</td>
<td>40 (23 - 91)</td>
<td>310 (138 - 617)</td>
<td>415 (191 - 902)</td>
<td>Kupper et al., 2008</td>
</tr>
<tr>
<td>China</td>
<td>Sludge</td>
<td>5.0 (0.4 - 58.7)</td>
<td>4.5 (&lt;3.4 - 69.7)</td>
<td>68.5 (&lt;1 - 1109)</td>
<td>94 ((2.1 - 1115)</td>
<td>Wang et al., 2007</td>
</tr>
<tr>
<td>Shanghai, China</td>
<td>Sludge</td>
<td>2.6 (0.48 - 5.6)</td>
<td>1.7 (0.25 - 3.7)</td>
<td>2370 (21.9 - 34900)</td>
<td>2430 (30.9 - 35300)</td>
<td>Yang et al., 2011</td>
</tr>
</tbody>
</table>
there was less than 4.7% of PBDEs discharged in the sewage effluent in the sewage treatment plant in Guangzhou, China (Peng et al., 2009). In California, the total PBDEs concentration in sludge was 116 times more than that in effluent (North, 2004). BDE-209 was the predominant congener in both the wastewater and sludge samples from China (Peng et al., 2009), and its concentration in the sludge from China could be 10 times higher than the western countries (Table 1.3). According to the worldwide studies of PBDEs in wastewater, PBDEs were not effectively degraded by the biological and chlorination treatments in wastewater treatment plants (WWTP) (Table 1.3). This may lead to the release of these non-degraded or partially degraded contaminants into the environment through discharging of effluent or disposal of sludge. It has been estimated about 2280 kg of PBDEs is discharged into the Pearl River with wastewater (Peng et al., 2009). Consequently, there is an urgent need to explore more advanced and efficient remediation technologies dealing with PBDEs in sewage.

1.7 Advanced Oxidation Processes (AOPs) for Remediation of Organic Pollutants

The ever-increasing concentration of emerging contaminants entering the environment and biotic samples has led to the interest of developing more effective treatment methods for the removal of these contaminants in wastewater.
Advanced oxidation processes (AOPs) are one of the potential methods for removing the trace levels of emerging chemicals in wastewater. During AOP, reactive free radicals (e.g. hydroxyl radicals) are produced which are able to reduce the toxicity and complexity of organic chemicals. Under suitable conditions (e.g. sufficient contact time, ultraviolet (UV) light, hydrogen peroxide (H₂O₂), etc), the pollutants may even be mineralized to the end-product as CO₂ (Gültekin and Ince, 2007). For example, it was reported that 92.5% of total organic carbon (TOC) was mineralized after advanced oxidation with UV light and hydrogen peroxide in 90 min (Ince et al., 2000). AOP can be applied for removing a wide range of emerging chemicals (e.g. acetaminophen, antipyrine, atrazine, caffeine and progesterone) in wastewater (Klamerth et al., 2009). It was reported that more than 90% of BDE-209 in acetonitrile was degraded by titanium dioxide (TiO₂) after 7.5 min of irradiation of UV radiation (Sun et al., 2009).

TiO₂ is a prominent photocatalyst used in AOP of organic pollutants. TiO₂ occurs in nature in three different forms: anatase, rutile and brookite. Both anatase and brookite will reform to rutile, which is the most common existing form, after heating (Greenwood et al., 1984). Nano-sized TiO₂ exerts photocatalytical function more efficiently than the other sized particles (Jiang et al., 2008). TiO₂ has been reported to be able to carry out photoreductive debromination of
BDE-209 (Sun et al., 2009). Accordingly, it is possible to debrominate BDE-209 into lower molecular weight congeners by photocatalytic TiO$_2$. Previous studies all focused on the characterization of kinetics and mechanisms of photocatalytical degradation of PBDEs in organic solvents rather than water (Söderström et al., 2004; Sun et al., 2009). In addition, the use of UV light will pose a tremendous burden on the operation cost of the sewage treatment plant. Therefore, visible light will be applied in this study to investigate whether the cost of the photocatalytical degradation can be reduced. In addition, the optimal operation conditions will also be identified in order to enhance the efficiency of the degradation.

1.8 Phytoremediation Strategies of PBDEs in Soil

The intermediate and/or products from the removal of the parent contaminants achieved by AOPs may be more toxic (Araña et al., 2008). In addition, the application cost could be another concern in the application of AOPs for treatment of emerging contaminants (Comminellis et al., 2008). Consequently, phytoremediation was suggested for further treatment of the photocatalyzed effluent containing residual PBDEs (in lower molecular weight) in the present study. Phytoremediation can be defined as green-plant based remediation technology for wastewater, contaminated soil and atmospheric pollutants.
Phytoremediation involves phytostabilization, phytosimulation, phytoextraction, phytovolatilization and phytodegradation (Pilon-Smits, 2005). Through phytoremediation, contaminants can be destroyed, inactivated, or immobilized and changed into non-toxic forms (Horne, 2000). Being slower than physico-chemical processes, phytoremediation can be applied as a long-term remediation process (Cunningham et al., 1995).

1.8.1 Interactions between Soil, Plants, PBDEs and Arbuscular Mycorrhizal (AM) Fungi during Phytoremediation of PBDEs

1.8.1.1 The Bioavailability of Organic Contaminants in Soil

The bioavailability of organic contaminants is an essential factor for phytoremediation, as this will govern the ability of organic contaminants to be taken up and metabolized by plant or plant-associated microbes (Alkorta and Garbisu, 2001). Bioavailability depends on the chemical and physical properties of the compound (Marschner, 1999), soil properties (organic matter content, pH, and clay content and type) (Cunningham, 1995) and the contact time of the contaminant with soil (Reid et al., 2000). In soil uptake of non-polar contaminants, partition into soil organic matter (SOM) is dominated due to the strong suppression of adsorption on mineral matter by water (Chiou et al., 2001). For the properties of contaminants, bioavailability can be interpreted by hydrophobicity
and volatility which are expressed as the octanol: water partition coefficient (log $K_{ow}$) and the Henry’s law constant (Hi) (Pilon-Smits, 2005). Contaminants that are highly hydrophilic (octanol-water partition coefficients, log $K_{ow} < 0.5$) cannot be sorbed by roots and/or through plant membranes (Briggs et al., 1982). Thus they may leach out of the root zones, which may require containment for remediation (Cunningham, 1995). Highly hydrophobic contaminants (log $K_{ow} > 3.0$) are more readily bound to the roots surfaces (Cunningham, 1995). Most PBDE congeners have a log $K_{ow}$ value > 3.0, implying a tendency of binding to the root surface (US EPA, 2008a; US EPA, 2008b; US EPA, 2008c).

Henry’s law constant is an indication of the inclination of a compound partitioning to air relative to water (Davis et al., 2003). Contaminants with Hi > $10^{-4}$ refers to a tendency to move into the air spaces between soil particles, whereas Hi < $10^{-6}$ a tendency to enter soil water. The contaminants are mobile in both soil water and air when the Hi values are between $10^{-6}$ and $10^{-4}$. Contaminants can move freely in both air spaces and soil water (Pilon-Smits, 2005). Aging is another obstacle for the phytoremediation of contaminants. Long periods of contact between contaminants and soil particles will lead to more sorption of contaminants onto the soil particles and may thus decrease the bioavailability of the contaminants. The contaminants become less mobile and
thus hinder the transformation (Hatzinger and Alexander, 1995). If these contaminants are reluctant to interact with other living organisms (e.g. arthropods or herbivores), or they may not be easily leached out, or not easily enter into the gastro-intestinal tract of human through accidental ingestion, phytostabilization can be applied (Cunningham, 1995).

1.8.1.2 The Roles of Plant in Phytoremediation of PBDEs-Contaminated Soil

Phytoremediation of organic contaminants can occur in two different ways, which are direct phytoremediation and phytoremediation ex planta (Alkorta and Garbisu, 2001). Direct phytoremediation strategies remove the contaminants through plant uptake, metabolism and/or accumulation (Schnoor et al., 1995). Phytoremediation ex planta refers to the strategies based on secretion by plants in root exudates, which support the growth of fungal and bacterial communities in the rhizosphere (Alkorta and Garbisu, 2001). Plants can be used in restoring contaminated soil due to two significant reasons. First, they can transport the contaminants from soil, with subsequent metabolism and assimilation after uptake. More importantly, the symbiosis between the plants and rhizosphere plays an essential role in removing organic pollutants (Leyval et al., 2002). The plants exude organic compounds and support the growth of pollutants degrading microbes (Smith and Read, 2008). The microbes provide nitrogen and
phosphorous, and serve as bio-control agents which inhibit the toxic effects of fungi in return to the plants (Neilson and Allard, 2008).

Direct uptake of organic contaminants in soil by plant is highly limited by the bioavailability and uptake mechanisms of the contaminants concerned (Salt et al., 1998). There has been extensive study about direct uptake of some organic contaminants, such as moderately hydrophobic pesticides and herbicides (Chu et al., 2006a; Kawahigashi, 2009). There are also a few studies about plant uptake of PBDEs, which are relatively more hydrophobic than pesticides and herbicides (log $K_{ow} = 5.52$ to 9.97) (US EPA, 2010; ATSDR, 2004b). The fate of the contaminants in the soil-plant system is essential to the understanding about the phytoremediation process. Mueller et al (2006) investigated the abiotic sorption and plant uptake of a commercial penta-BDE mixture and found that PBDEs could be accumulated in yellow gold rush and small round red cherriette. The partitioning of organic contaminants into roots and aboveground tissues depends on the hydrophilic properties of the contaminants in concern (Alkorta and Garbisu, 2001). Since PBDEs are hydrophobic substances and thus they inclined to accumulate in the roots which possess a higher lipid content than the aboveground plant tissues. A study showed that the translocation factors (TF, $C_{\text{shoot}}/C_{\text{root}}$) of BDE-209 in all six tested plants (Italian ryegrass, alfalfa, pumpkin, summer
squash, maize, and radish) were smaller than one, implying more BDE-209 was accumulated or taken up by roots than the aboveground tissues (Huang et al., 2010).

A wide spectrum of organic contaminants is degraded more readily in rhizosphere than in soil alone (Gao et al., 2006; Chekol et al., 2004). The enhanced degradation in rhizosphere will be discussed as the following. First, the carbon flow in the rhizosphere greatly enhances the microbial activity and thus the degradation of contaminants (Meharg, and Cairney, 2000). Second, roots of the host plant may produce some secondary metabolites, which may be the analogues of the contaminants (Meharg, and Cairney, 2000). Therefore, the micro-organisms may use the contaminants for growth as the energy source and co-metabolize them when consuming the secondary metabolites (Meharg, and Cairney, 2000). Third, the root network may change the chemical/physical structure of soil eventually altering the partitioning of the contaminants (Meharg, and Cairney, 2000). Finally, the transpiration streams may promote the diffusion of contaminants into the rhizosphere (Meharg, and Cairney, 2000).

Table 1.4 lists the advantages and limitations of phytoremediation. Plants help to reduce soil erosion by wind, lower the possibility of human contact to contaminated soil and also diminish environmental impacts (Cunningham et al.,
Table 1.4 Advantages and limitations of phytoremediation

<table>
<thead>
<tr>
<th>Advantages</th>
<th>Limitations</th>
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<tr>
<td>relatively cost-effective than chemical methods&lt;sup&gt;a&lt;/sup&gt;</td>
<td>requires oxygen, water and nutrients for plant growth&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>more natural, environmental friendly and socially accepted technology&lt;sup&gt;a&lt;/sup&gt;</td>
<td>removal abilities depends on soil property, climate and toxicity level&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>non-intrusive&lt;sup&gt;a&lt;/sup&gt;</td>
<td>limited to the soil depth the roots can reach&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>solar-driven&lt;sup&gt;b&lt;/sup&gt;</td>
<td>depends on bioavailability of pollutant&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>in situ&lt;sup&gt;b&lt;/sup&gt;</td>
<td>highly water-soluble contaminants may leach outside the root zone&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>easy to implement&lt;sup&gt;b&lt;/sup&gt;</td>
<td>slower than physio-chemical methods&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

Alkorta and Garbisu, 2001<sup>a</sup>, Pilon-Smits, 2005<sup>b</sup>, Cunningham et al., 1995<sup>c</sup>
Plants can further modify the water regime of soil and reinforce the soil strength. They thus reduce the surface water run-off and resist the soil contaminant leakage from rain water washing (Angers and Caron, 1998). Nevertheless, the soil properties, climate, contaminants concentration and properties can all significantly interfere with the phytoremediation process (Pilon-Smits, 2005).

1.8.1.3 The Roles of AM Fungus in Phytoremediation of Organic Contaminated Soil

Mycorrhizas are ubiquitous root-fungus symbioses between soil fungus and roots, which include three groups: ectomycorrhizas, ericoid mycorrhizas and arbuscular mycorrhizas (AM) (Joner and Leyval, 2003). AM can form on most herbaceous plants, shrubs and temperate trees (Smith and Read, 2008). Arbuscular mycorrhizas are endomycorrhizas, with fungi enter into root cells during colonization. It is opposed to ectomycorrhizas in which fungi merely envelop the epidermis (Joner and Leyval, 2003).

As stated previously, phytoremediation of organic contaminants may involve phytoremediation ex planta or rhizodegradation (degradation in rhizosphere) and phytodegradation (degradation in the plants) (Alkorta and Garbisu, 2001). Mycorrhizas play a significant role in rhizodegradation of organic contaminants,
assisting the host plant to acquire nutrition outside the rhizosphere, where the hyphae absorb mineral nutrients (Smith et al., 2001). Arbuscular mycorrhizal fungi have been previously manifested to increase the biomass and grains yield of rice (Secilia and Bagyaraj, 1992). Many studies have tried to investigate the reason behind of this phenomenon. The fungi obtain carbon from the host plants and return back for the plants with phosphate and other mineral nutrients from soil (Harrison and van Buuren, 1995). Considering the symbiotic phosphate uptake of *Oryza sativa* (rice), which is the first crop with complete sequenced genome, by *Glomus intraradices*, attempt has been made to identify the transporter genes involved and indicated that the *Oryza sativa* phosphate transporter gene OsPT11, which encodes a functional inorganic (ortho)phosphate transporter, was specifically induced during the arbuscular mycorrhizal symbiosis (International Rice Genome Sequencing Project, 2005; Paszkowski et al., 2002). This explained why arbuscular mycorrhizal (AM) fungi enhanced the availability of inorganic (ortho)phosphate transporter to plants (Paszkowski et al., 2002). In addition, the contribution of AM fungi in phytoremediation of some organic pollutants (e.g. polycyclic aromatic hydrocarbons (PAHs)) has been reported. Nevertheless, there is limited information on phytoremediation of PBDEs with AM fungi, so the studies involved PAHs which have similar characteristic with PBDEs can be used
as references. A study showed that AM fungi might have contributed to the establishment of plants in PAH-contaminated soil (Leyval and Binet, 1998). In addition, extraradical mycorrhizal hyphae can also exert significant effects on rhizodegradation through extension of rhizosphere, by assisting nutrient supplementation and promoting enzymatic activities in soil outside the rhizospheric zone (Joner and Leyval, 2003). The hyphae also possess the degradation potential that they allocate carbon to the microbial population in soil compartment (Joner and Leyval, 2003). In a study on the potential use of AM hyphae to mediate uptake of PAHs by the roots of ryegrass (Lolium multiflorum Lam.), it was found that the partition coefficients ($K_d$) of PAHs by mycorrhizal hyphae were 270–356% greater than those by roots (Gao et al., 2010). This further confirmed the excellent ability of hyphae to absorb organic contaminants, such as PBDEs. Consequently, the mycorrhiza may probably affect the remediation of PBDEs in soil in an indirect manner.

1.8.2 Application of Phytoremediation for the Removal of PBDEs in Soil under the Influence of AM Fungi

Extensive research has been conducted on the phytoremediation of various contaminants, such as PCBs and PAHs (Chekol et al., 2004; Gao et al., 2010). Nevertheless, there is still lack of thorough research in the phytoremediation of
PBDEs, especially with the application of AM fungi. To the best of our knowledge, there is currently only one related study conducted on degradation of BDE-209 in the rhizosphere of ryegrass, with inoculated AM fungi. A negative correlation ($p < 0.001$, $r^2 = 0.66$) between the residual BDE-209 concentration in the soil and microbial biomass in the soil was observed, providing the evidence of rhizodegradation of BDE-209 by soil microbes with the help of AM fungi (*Glomus mosseae*) (Wang et al., 2011a). There are plenty of aspects which can be further developed from this study in the phytoremediation of BDE-209. The influences of other fungi in the *Glomus* genus on phytoremediation of PBDEs can also be taken in account. For example, *Glomus intraradices*, which has been reported to contribute positively to the roots of the host plant in PAH tolerance (Verdin et al., 2006), would be an alternative of *Glomus mosseae*, which has been used in Wang et al. (2011a). Comparison of the effects of different varieties of AM fungi can also provide some clues in the mechanisms of the dissipation of PBDEs in the rhizosphere. Furthermore, rice (*Oryza sativa L.*), which is the first crop to have its entire genome sequenced (International Rice Genome Sequencing Project, 2005), can be applied in the study of phytoremediation of PBDEs, so as to provide new information in transgenic technology for enhancement of contaminants removal abilities of plants. Furthermore, Su and Zhu (2007) suggested the
potential of *Oryza sativa* (rice) in uptake of six organic contaminants (dinitrobenzene (DNB), dinitrotoluene (DNT), lindan (LIN), 1,2,3-trichlorobenzene (TCB), phenanthrene (PHN) and pyrene (PYR)), and pointed out that the relatively more hydrophobic compounds in the group, such as PHN (log $K_{ow}$ = 4.46) and PYR (log $K_{ow}$ = 5.18) transport mainly through the apoplastic pathway in rice seedlings. Another study also stated the ability of rice plants in remediation of PAHs contaminated soils, and found that the concentrations of PHN and PYR in rice roots increased proportionally with those in soil pore-water (Su and Zhu, 2008). Chu et al. (2006) examined the accumulation and distribution of DDT and PCBs in rice plants and elucidated the ability of rice plants in removal of these two contaminants in hydroponic culture solution. DDT and PCBs have similar chemical and physical characteristic as PBDEs and thus it proposed the potential use of rice plants in phytoremediation of PBDEs (Rahman et al., 2001).

1.9 Combined TiO$_2$-Photocatalysis and Phytoremediation

Extensive research has been conducted on advanced oxidation processes (AOP) degradation and phytoremediation of organic pollutants. Nonetheless, both the technologies have drawbacks and limitations which may hinder the practical application of degradation of PBDEs. For instance, the installation cost of AOPs facilities is reasonable but the operational cost and energy consumption are high
Phytoremediation is a relatively slow process, with time and space the crucial factors in restoration of contaminated soil (Cunningham, 1995). More importantly, the by-products and/or metabolites derived from these two processes may be even more toxic than the parental compounds (Arslan-Alaton et al., 2008; Alkorta and Garbisu, 2001). Consequently, the application of either of these two technologies alone is not cost-effective and environmental-friendly enough for the treatment of PBDEs in sewage. For this reason, a combination of these two technologies has recently been proposed. A combination of a photocatalytic technique and phytoremediation is a very innovative technology in treatment of contaminants in wastewater (Antoniadis et al., 2007). The combined system employs a synergistic action of both heterogeneous TiO\textsubscript{2} photocatalysis and a wetland reactor so as to improve water quality by photocatalysis and a natural process (Antoniadis et al., 2010). There have been several research papers concerning the feasibility of treatment of wastewater by the hybrid technique (combination of photocatalytic oxidation with constructed wetlands) published recently. Antoniadis et al. (2007) used the Photo-Fenton reagent for the reduction of the organic load in sewage and the treated effluent was then purified by constructed wetland. Three years later, the same research group conducted a similar study with heterogeneous photocatalytic
TiO$_2$ P-25 Degussa, and showed that the final effluent of the integrated system was free of *E. coli* (Antoniadis et al., 2010). Mahne et al. (2012) also showed that decolouration of textile wastewater was accelerated with constructed wetland pretreatment and the substantial photocatalytic oxidation process could promote COD removal up to 45%. Another study indicated that complete detoxification of 4-nitrophenol (4NP) (up to 200 ppm) was achieved by the combination of solar TiO$_2$-photocatalysis (6 h) with constructed wetlands (16 h) (Herrera-Melián et al., 2012). In an attempt to investigate treat and reclaim wastewater using photocatalysis and constructed wetlands, it was observed that the combined system enhanced the removal efficiencies of precursors of disinfection by-product (DBP). It also reduced the formation of simulated distribution system disinfection by-product (SDS-DBP) in the final out-flow water, which can even reach the requirement standards of drinking water (Chen et al., 2011). All the results from the above studies indicate that the combined systems are more effective in the reduction of contaminants and disinfection of pathogens than either of the two methods alone. This demonstrated the potential of the combined TiO$_2$-photocatalysis and constructed wetland system in the elimination of harmful substances in municipal sewage.

1.10 Research Aims and Objectives
In the light of the background provided, the present study aims to investigate the remediation of PBDEs by photocatalytic degradation and phytoremediation. A combined remediation system will be developed with advanced oxidation processes (AOPs) pre-treatment and constructed wetland. It was hypothesized that TiO$_2$ could degrade BDE-209 under visible light, and rice plants were able to uptake and accumulate PBDEs. More importantly, the combined photocatalytic degradation and phytoremediation system was hypothesized to promote the removal efficiency of PBDEs. More specifically, the objectives of the study were:

1. To investigate the ability of photocatalyst (TiO$_2$) to degrade BDE-209 and render them more bioavailable products to *Oryza sativa*.

2. To determine the optimal operation parameters for the photocatalytic degradation of BDE-209.

3. To evaluate the fate and translocation of BDE-47, BDE-99 and BDE-209 in plants.

4. To investigate the uptake mechanisms of PBDEs by a rice cultivar.

5. To identify the interactions of AMF and plants on PBDEs dissipation.

6. To examine the effects of changes of pHs on phytoremediation of BDE-47, BDE-99 and BDE-209.

7. To testify the efficiency of the removal of BDE-209 after combining the
photocatalytic degradation and phytoremediation system.

1.11 Significance and Contribution of This Study

To the best of our knowledge, this is the first investigation on the feasibility of remediating PBDEs by combining the photocatalysis and phytoremediation technologies. This may contribute to the knowledge base with regards to a better understanding of the application of a hybrid system in remediation of emerging contaminants in sewage. More importantly, this may provide a significant reference for the development of advanced sewage treatment systems in the future.

This is also one of very few studies investigating the effect of AM fungi on phytoremediation of PBDEs by rice plants. The use of constructed wetlands provides essential information on the fate and uptake mechanism of PBDEs in the soil-plant system, which may also enrich our knowledge of in situ restoration of PBDEs contaminated land by plants.

1.12 Framework

The conceptual framework of this study is illustrated in Fig. 1.2, which highlights the four stages of the study. It includes the investigation of the feasibilities of photocatalytical degradation and phytoremediation of PBDEs. The effects of different operation parameters (pH, humic acid (HA) content and crystalline forms of TiO₂) and various planting conditions (pH and arbuscular
Figure 1.2 The conceptual framework of the present study.
mycorrhizal (AM) fungi) on the efficiency of the PBDEs degradation will be examined. Most importantly, the efficiency improvement after combining the photocatalytic degradation and phytoremediation system will be investigated.
CHAPTER 2
CHARACTERIZING THE OPTIMAL OPERATION OF PHOTOCATALYTIC DEGRADATION OF BDE-209 BY NANO-SIZED TiO$_2$

2.1 Introduction

Since the 1970s, the demand of brominated flame retardants has been increasing (Wang et al., 2007). The annual global production of polybrominated diphenyl ethers (PBDEs) increased from 40,000 tons (Arias, 1992), to around 67,000 tons between 1992 to 2001 (BSEF, 2006). Asian countries shared about 40% of the global demand of PBDEs (BSEF, 2006). PBDEs are an important class of brominated flame retardants with a high production rate. They are generally used in polymer and textile products as additive flame retardants (de Wit, 2002; Rahman et al., 2001). PBDEs added resins or polymers are also common components of electrical appliances, contributing to the release of PBDEs from electronic waste (e-waste) (WHO, 1994). Due to their persistent, lipophilic and bioaccumulating characteristics, the concern on their uses has been increasing since the 1990s (de Wit, 2002). PBDEs were reported to be potential endocrine disruptors (Lema et al., 2008) and neurotoxicants (Goodman, 2009).
In the past decade that the life-span of electronic appliances is becoming shorter and shorter due to the advanced technology, many developed countries export e-waste to developing countries in East and Southeast Asia for recycling. Illegal and inappropriate dumping and recycling of e-waste are believed to be two major sources of PBDEs contamination in the environment (Wang et al., 2005). For example, PBDEs (2720–4250 ng/g dry weight) were found in soils from an acid leaching site (using strong acids to recover metals from electronic boards) of Guiyu (Leung et al., 2007). Although the European Union (EU) has banned the usage of all penta-BDE and octa-BDE in the EU market since 2003 (EU, 2001), deca-BDE is still in use in electrical and electronic equipments and may lead to contamination via acid leaching (Leung et al., 2007). It has been revealed that the soils in Guiyu, China (an e-waste recycling site) are polluted by PBDEs in which BDE-209 is the dominant congener and occupied 35% to 82% of the total PBDEs content (Leung et al., 2007), BDE-209 was therefore chosen as the tested chemical in this study. Tertiary-level wastewater treatment plants of modern wastewater treatment are insufficient in removing PBDEs from wastewater. BDE-47, BDE-99, and BDE-209 were the major congeners identified in sludge and effluents from a tertiary sewage treatment facility that discharges into natural waters (North, 2004). PBDEs in wastewater may adsorp onto wastewater sludges
while some may enter natural water bodies resulting in a large PBDEs flux into receiving waters and thus, posing potential hazards to drinking water sources and fisheries resources (Rayne and Ikonomou, 2005b), and there is a need to investigate effective removal of these pollutants, so as to protect wildlife and human health.

To cope with an uprising application of PBDEs and its release into the environment, different remediation technologies should be developed. Plant uptake and dissipation of PBDEs by plants were investigated by Italian ryegrass, pumpkin, and maize. The reduction rates of the total PBDEs in the soils were low (13.3% to 21.7%). Although it is a relatively environmental friendly method for remediation of PBDEs, but it usually takes longer time (e.g. 60 days) and requires more space (Huang et al., 2011). Photolytic debromination was attempted for remediation of deca-BDE. Nevertheless, the half-life (150–200 hr) of using artificial UV light was not ideal for treatment of highly PBDEs-contaminated soil (Söderström et al., 2004). Therefore, more advanced remediation technology such as advanced oxidation processes (AOPs) was developed for removing trace levels of emerging chemicals (e.g. acetaminophen, antipyrine, atrazine, caffeine and progesterone) in wastewater (Klämerth et al., 2009). It was reported that more than 90% of BDE-209 in acetonitrile was degraded by TiO₂ after 7.5 min of
irradiation of UV radiation (Sun et al., 2009). During AOP, reactive free radicals (e.g. hydroxyl radicals) are produced and are able to reduce the toxicity and complexity of organic chemicals. Under suitable conditions, the pollutants (e.g. total organic carbon) may even be mineralized to the end product as CO₂ (Gültekin and Ince, 2007). For example, it was reported that 92.5% of total organic carbon was mineralized after advanced oxidation with UV light and hydrogen peroxide in 90 min (Ince and Apikyan, 2000). TiO₂ is a prominent photocatalyst used in AOP for treating organic pollutants. Nanosized TiO₂ is even more effective in photocatalytical activity than other sized-TiO₂ (Jiang et al., 2008). Previous studies all focused on the characterization of kinetics and mechanisms of photocatalytical degradation of PBDEs in organic solvent and on natural matrices other than water (Söderström et al., 2004; Sun et al., 2009).

Photocatalytical degradation of emerging contaminants (e.g. synthetic hormones) by TiO₂ has been investigated (Ohta et al., 2002; Sun et al., 2009; Panchangam et al., 2009) but very few studies focused on the degradation of PBDEs by TiO₂. Photoreductive debromination of BDE-209 by TiO₂ under UV light, via debromination pathway has been reported (Sun et al., 2009). However, high operational costs are associated with the degradation technology of PBDEs by TiO₂ under UV light and may not be efficient enough to treat large-scale
loadings of wastewater in modern cities. Consequently, there is an urgent need for characterization of an ideal removal system of PBDEs, especially BDE-209 by TiO$_2$, which can provide information for further developments of advanced wastewater treatment plant.

The present study was aimed at investigating the ability of photocatalyst (TiO$_2$) to degrade BDE-209. The time course of BDE-209 degradation by TiO$_2$ was studied. In addition, the efficiencies of photocatalytical degradation of BDE-209 by TiO$_2$ at different pHs, humic acid concentrations and crystalline forms of TiO$_2$ were examined for the identification of optimal operation parameters, providing further information for future advanced remediation of wastewater containing PBDEs.

2.2 Materials and Methods

2.2.1 Materials

BDE-209 was obtained from Dr. Ehrenstorfer (Germany), while mass-labeled ($^{13}$C$_{12}$) surrogate and internal standard solutions of PBDEs were purchased from Wellington Laboratories Inc. (Canada). TiO$_2$, mixture of rutile and anatase (nanopowder < 100 nm particle size, 99.5% trace metals basis), TiO$_2$, anatase (nanopowder < 25 nm particle size, 99.7% trace metals basis) and TiO$_2$, rutile (nanopowder < 100 nm particle size, 99.5% trace metals basis) were
obtained from Sigma-Aldrich (USA).

2.2.2 Reactive Oxygen Species (ROS) Production by TiO$_2$ with BDE-209

Nano-sized TiO$_2$ solution (1% mixture of anatase and rutile crystalline forms) was prepared in 0.1% dimethyl sulfoxide (DMSO) in pH 7 buffer solution. The solution was then sonicated for 35 min in an ultrasonic cleaner (Branson Model 3510, 40 kHz). The negative control did not contain TiO$_2$ and the particle control was added with nano-sized SiO$_2$ instead of TiO$_2$. BDE-209 in 0.1% DMSO (75 ppb) was spiked into the solutions. Dichlorofluorescein diacetate (H$_2$DCF-DA) (Invitrogen D-399) is a common probe used for detecting hydroxyl radicals (LeBel et al., 1992). The hydroxyl radicals generated by TiO$_2$ and SiO$_2$ were measured by dichlorofluorescein (DCF) assay. First, 2',7'-dichlorofluorescin diacetate (DCFH-DA) (2.5 mmol/L) was hydrolyzed in 0.01 mol/L sodium hydroxide (NaOH) for 30 min in the dark at room temperature (25°C) for the preparation of DCFH stock solution. The mixture was then neutralized with 0.1 mol/L PBS to pH 7.4, followed by centrifugation at 3000 r/min for 10 min. The supernatant was removed and resuspended in 500 μL DMSO. DCFH solution (25 μmol/L) was added to 500 μL of the samples for 30 min. Finally, 200 μL of the solutions were injected into 96- well plates for fluorescence measurements by a microplate reader ($\lambda_{\text{excitation}} = 498$ nm; $\lambda_{\text{emission}} = 522$ nm) (TECAN infinite F200)
(Foucaud et al., 2007). The productions of ROS in treatment samples were indicated as the ratios between relative fluorescence units (RFU) of the treatments to those of controls.

Interaction between the ROS production by TiO$_2$ during different time intervals was studied. The control and treatment solutions were placed in a fluorescent lamp chamber (103 μmol/m$^2$/sec) and shaken for 4 hr, and 1, 2, 3, 4, 5 and 6 days using a shaker. After that, 500 μL of the samples were sampled and ROS measured according to the procedure stated previously (Foucaud et al., 2007).

The ROS productivity of TiO$_2$ on BDE-209 at different pH levels was examined by adjusting the control and treatment solutions to pH 4, 6, 7, 8 and 12 with HCl (1 mol/L) and NaOH (1 mol/L), following the work by Zhang et al. (2008). The solutions were shaken in the light chamber for 4 hr using a shaker. After that, 500 μL of the samples were extracted and ROS measured according to the procedure stated previously (Foucaud et al., 2007).

The effect of humic acid concentration on ROS productivity of TiO$_2$ on BDE-209 was analyzed by adding various humic acid concentrations (5, 10, 20, 40 mg/L) according to Zhang et al. (2008), into the control and treatment solutions. The stock solution was prepared by dissolving 100 g of humic acid in
0.1 mol/L NaOH solution, followed by dilution with 1000 mL distilled water. Desired testing concentrations were then prepared by further dilutions with distilled water. The solutions were shaken in a fluorescent lamp chamber for 4 hr. After that, 500 μL of the samples were extracted and ROS measured according to the procedure stated previously (Foucaud et al., 2007).

TiO₂ occurs in nature as three different forms, anatase, rutile and brookite (Greenwood and Earnshaw, 1984). The effects of distinct structures of TiO₂ (anatase, rutile, and mixture of anatase and rutile) on ROS productivity were evaluated by adding different forms of TiO₂ into the control and treatment solutions. The solutions were shaken in a light chamber for 4 hr. After that, 500 μL of the samples were extracted and ROS measured according to the procedure stated previously (Foucaud et al., 2007).

2.2.3 Photodegradation of BDE-209 by Nano-sized TiO₂

Nano-sized TiO₂ solution (1% mixture of anatase and rutile crystalline forms) was prepared in 0.1% DMSO in pH 7. The solution was then sonicated for 35 min in an ultrasonic cleaner (Branson Model 3510, 40 kHz). The negative control did not contain TiO₂ and the particle control was added with nano-sized SiO₂ instead of TiO₂. BDE-209 in 0.1% DMSO was spiked into the solutions with different operation conditions (pH, humic acid concentrations and crystalline forms of TiO₂)
to final concentration of 75 µg/L. The solutions were shaken in the light chamber (400–530 nm) for 6 days.

The residual products and BDE-209 left after the photodegradation were extracted by liquid-liquid extraction and quantified by a gas chromatography-mass spectrometry (GC-MS). The mixture solutions mentioned above (20 mL) were shaken with 20 mL dichloromethane (DCM) in a separation funnel in order to extract PBDEs. The lower immiscible part of solution (PBDEs dissolved in 20 mL of DCM) was then drained into a round bottle flask. This step was repeated for 3 times and totally 60 mL of DCM was obtained from each treatment. The extracts were then concentrated by a rotary evaporator and replaced by hexane (nearly 2 mL) (US EPA, 1996a). GC-MS analysis of the samples was determined according to US EPA standard method 1614 (US EPA, 2007).

2.2.4 Quality Control

Extraction and analysis were conducted in dark, to minimize the exposure to light. A procedure blank was included in each batch of extraction. The extracts were analyzed using an Agilent 7890A GC-MS instrument connected with an Agilent 5975C inert MSD triple-axis detector (Agilent Technologies, USA). The limit of detection (LOD), which was calculated as a mean of the background
signal plus three times the standard deviation in the blank samples, was 0.5 to 1 μg/L for BDE-3 to BDE-191, 4 to 5 μg/L for BDE-197 to BDE-206, 100 μg/L for BDE-209. Surrogate standards were added into samples prior to extraction and the recoveries ranged from 76.1 to 112%.

2.2.5 Data Analysis

The statistical analyses of the data were performed by using the SPSS version 16 software package for Windows. The statistical values were all calculated for triplicates. A 95% confidence limit (p < 0.05) was applied for the indication of significant differences between samples.

2.3 Results and Discussion

2.3.1 Time Course of BDE-209 Degradation

Nano-sized TiO₂ significantly enhanced the degradation of BDE-209 (HA/visible light), compared with the control (p < 0.05) (Fig. 2.1a). The photocatalytic effect was only shown under the light but not in the dark control, where the reduction rate remained at 6% and 7% in control and TiO₂ treatment respectively (Fig. 2.1a). Figure 2.1b illustrates the photocatalytical degradation of BDE-209 by TiO₂ during the 14 days. The reduction rate of BDE-209 increased with time at the beginning until day 6 (71.1%) and then leveled off at day 14 (72.4%). With initially only BDE-209 in the solution, the photocatalytical
degradation of BDE-209 in the current study actually followed the first-order rate law in the beginning 6 days (Fig. 2.1c) (Equation 2.1).

\[ y = 1.88 - 0.0945t \]  \hspace{1cm} (2.1)

where, \( y \) is the log concentration of BDE-209 and \( t \) (day) is the reaction time. According to Equation 2.1, the half-life for BDE-209 degradation in this study was 3.05 days. There is a lack of information concerning the natural degradation pathway of BDE-209 in water as no prior studies were conducted to investigate its degradation kinetics in water under visible light. However, the results of natural photodegradation of BDE-209 in other environmental matrices by sunlight from previous studies may provide some clues for interpreting the results of this study (Table 2.1). It indicated that the half-life of photocatalytical degraded BDE-209 in this study was much lower than those of naturally degraded BDE-209 adsorbed onto other natural matrices (Table 2.1). This suggested that photocatalytic TiO\(_2\) may enhance the photodegradation of BDE-209 and shorten their half-lives. It was suggested that reactive oxygen species may play a significant role in photocatalytical degradation of PBDEs (Raff and Hites, 2006). The present results showed that the hydroxyl radicals produced by TiO\(_2\) increased from 4 to 8 hr and then decreased until day 6. Although there was a drop after 8 hr, the levels of hydroxyl radicals still remained at double of the control level (Fig. 2.1d).
Figure 2.1 Photocatalytical degradation of BDE-209 and induction of ROS by TiO₂. (a) Degradation rates of control with no nanoparticles and treatment with TiO₂; (b) Degradation of BDE-209 and formation of different congeners by TiO₂ at different time intervals; (c) Photocatalytical degradation rate of BDE-209 by TiO₂; (d) Influence of time intervals on hydroxyl radicals production by nano-sized TiO₂. Points with the same letter at the top were not significantly different (p > 0.05) according to one-way ANOVA test.
Table 2.1 Degradation of BDE-209 in different matrices with or without TiO$_2$

<table>
<thead>
<tr>
<th>Solid matrixes/solvents</th>
<th>Half-lives ($t_{1/2}$)</th>
<th>Rate constant ($k$)</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Sunlight</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sediment</td>
<td>990 days</td>
<td>0.0007 ± 0.0003 day$^{-1}$</td>
<td>Ahn et al., 2006</td>
</tr>
<tr>
<td></td>
<td>80 hr</td>
<td></td>
<td>Söderström et al., 2004</td>
</tr>
<tr>
<td></td>
<td>81 hr</td>
<td></td>
<td>Sellstrom et al., 1998</td>
</tr>
<tr>
<td>Sand</td>
<td>37 hr</td>
<td></td>
<td>Soderstrom et al., 2004</td>
</tr>
<tr>
<td></td>
<td>533 hr</td>
<td></td>
<td>Hua et al., 2003</td>
</tr>
<tr>
<td><strong>UV</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.1% TiO$_2$ in hexane</td>
<td>&lt; 10 min</td>
<td>0.12 ± 0.0049 min$^{-1}$</td>
<td>Sun et al., 2009</td>
</tr>
<tr>
<td>0.1% TiO$_2$ in acetonitrile</td>
<td>2.1 min</td>
<td>0.33 ± 0.02 min$^{-1}$</td>
<td>Sun et al., 2009</td>
</tr>
<tr>
<td>methanol</td>
<td>51 min</td>
<td></td>
<td>Eriksson et al., 2004</td>
</tr>
<tr>
<td><strong>Visible light (400–530 nm; 103 µmol/m$^2$/sec)</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.1% DMSO</td>
<td>4.05 days</td>
<td></td>
<td>This study</td>
</tr>
<tr>
<td>1% TiO$_2$ in 0.1% DMSO</td>
<td>3.05 days</td>
<td></td>
<td>This study</td>
</tr>
</tbody>
</table>
Debromination of PBDEs may be attributed to the production of hydroxyl radicals. When the TiO$_2$ is photoexcited, an electron-hole pair is formed. The holes are scavenged by the hydroxyl groups of adsorbed water, yielding hydroxyl (•OH) radicals which add to the aromatic ring of BDE-209 or the reaction products. The addition of the hydroxyl radical weakens the aryl-Br bond leading to a subsequent cleavage, and the hydroxyl group is then replaced by the bromine atom. The hydroxyl radicals will then generate a series of oxidation in the aromatic ring system (An et al., 2008).

2.3.2 Effect of pH Levels, Concentrations of Humic Acids, and Crystalline Forms of TiO$_2$ on Photocatalytical Degradation of BDE-209

In general, the majority of photocatalytical degradation products after treatment with nano-sized TiO$_2$ under three different operational conditions were dominated by tetra- and penta-BDEs (Fig. 2.2a). They contributed to 25.7% and 33.7%, respectively of the photocatalytical degradation products at pH 12 (Fig. 2.2a). Photocatalytic reaction takes place on the surface of the semiconductor photocatalyst, TiO$_2$. Hence, the pH level of the solution is important to the photocatalytic function of TiO$_2$, because it determines the surface charge property and dispersion of TiO$_2$ (Haque and Muneer, 2007). Under acidic conditions, TiO$_2$ will be protonated as TiOH$_2^+$, creating a positive charge on the surface of the
Figure 2.2 Degradation profiles of BDE-209 by TiO$_2$ in 0.1 % DMSO under different operating conditions. (a) After photocatalytical degradation by TiO$_2$ at different pH levels, C: control; S: particle control using SiO$_2$; T: treatment using TiO$_2$; (b) After photocatalytical degradation by TiO$_2$ in different concentrations of humic acid, C: control; S: particle control using SiO$_2$; T: treatment using TiO$_2$; (c) After photocatalytical degradation by TiO$_2$ in different crystalline forms.
catalyst. In contrast, TiO$_2$ will be deprotonated as TiO$^-$ under alkaline conditions, creating a negative charge on the surface of the catalyst (Sun et al., 2006). Since the majority of the TiO$_2$ particles are monocharged at these extreme pH levels, they will repel each other and become dispersed. This can probably increase the effective surface area in contact with BDE-209 and thus enhance the degradation at pH 12.

Figure 2.2b shows the distribution of PBDEs products from photocatalytical degradation of BDE-209. A higher proportion of BDE-209 was reduced in humic acids, concentrations ranging from 5–20 mg/L. Penta- (40.6%) and tetra-BDEs (38.6%) were the most dominant congeners observed in 20 mg/L of humic acids. It has been suggested that the adsorption between humic acids and TiO$_2$ is pH-dependent (Cho and Choi, 2002; Li et al., 2002). Adsorption of humic acids with TiO$_2$ increased at lower pH levels. At pH 7, adsorption of humic acids was near saturation at 10 mg/L of the initial concentration of humic acids. An increase of the initial concentration to 25 mg/L only contributed to a slight rise of adsorption isotherms (Cho and Choi, 2002). Therefore, the adsorption of humic acids with TiO$_2$ was concentration-dependent at pH 7. When the initial concentration went beyond 20 mg/L, adsorption between humic acids and TiO$_2$ declined and thus inhibited the photosensitization effect of humic acids on TiO$_2$. 
Accordingly, the photocatalytical degradability of BDE-209 also declined at 40 mg/L.

Figure 2.2c illustrates the percentage distribution of PBDE congeners after being photocatalysed by TiO₂ in different crystalline forms. Again, tetra- (33.3%) and penta-BDE (34.5%) were the most dominant congeners found in the degraded residues with the mixture of anatase and rutile. It has been reported that the crystalline forms of TiO₂ would affect the morphology and microstructure characteristics, and thus the photocatalytical activity of TiO₂ particles (Tayadea et al., 2007; Collins-Martínez et al., 2007). Anatase and rutile, the two basic catalytic crystalline forms of TiO₂, contributed to differences in microstructure, and thus varied in photocatalytical efficiencies. For example, anatase has a higher adsorptive ability towards organic compounds than rutile (Stafford et al., 1993). In addition, anatase also has a larger band gap than rutile (Mo and Ching, 1995). Due to the interplay of these characteristics, the mixture of anatase and rutile usually displayed more advanced photocatalytical effects (Bojinova et al., 2007). In mixed-phase TiO₂ catalysts, electrons are transferred from anatase to the electron trapping sites of rutile which is at a lower energy state. The recombination rate of anatase is thus lowered, resulting in more efficient BDE-209 was degraded in the mixture of anatase and rutile (residual portion = 17.1%).
2.3.3 Relations between ROS Production and Photocatalytic Degradation of BDE-209 under Different Operational Conditions

2.3.3.1 At Different pH Levels

Significant differences were observed between treatments with TiO$_2$ and the controls with SiO$_2$, indicating that the degradability of BDE-209 was mainly due to the photocatalytic activity, but not the nano-size of the nanomaterials. It was found that the relative fluorescence units (RFU) ratio of treatments over controls was the highest at pH 8 (3.98 ± 0.169) and pH 12 (4.20 ± 0.423) (Fig. 2.3a). This means that induction of the hydroxyl radicals was the highest at pH 8 and 12, and can be attributed to the higher concentration of hydroxide (OH$^-$) ions in the solution (Herrmann et al., 1993). Meanwhile, the production of BDE-47 (11.5 ± 1.10 nmol/L) and BDE-99 (6.82 ± 0.547 nmol/L), and the reduction percentage of BDE-209 (93% ± 1%) were the greatest at pH 12 (Fig. 2.4). There was a significant positive correlation between ROS induction and BDE-47 ($r = 0.652$, $p < 0.01$) and BDE-99 ($r = 0.835$, $p < 0.01$) production, which revealed that the generation of these congeners may be related to the increase of ROS.

Hydroxyl radicals were reported to be responsible for photolysis of organic pollutants (Peterson et al., 1991). The generation of hydroxyl radicals actually depends on the pH level of the solution. The productions of hydroxyl radicals in
Figure 2.3 Production of ROS by TiO$_2$ in 0.1% DMSO under different operating conditions. (a) Effect of pH levels on hydroxyl radicals production by TiO$_2$ (RFU ratio of treatment over controls); (b) Effect of concentrations of humic acid on hydroxyl radicals production by TiO$_2$ (RFU ratio of treatment over controls); (c) Effect of different crystalline structures of TiO$_2$ on hydroxyl radicals production by TiO$_2$ (RFU ratio of treatment over controls). Points with the same letter at the top were not significantly different (p > 0.05) according to one-way ANOVA test.
Figure 2.4 Photocatalytical degradation by TiO$_2$ in 0.1% DMSO with different pHs. (a) Concentration of BDE-47 in 0.1% DMSO; (b) Concentration of BDE-99 in 0.1% DMSO; (c) Reduction percentage of BDE-209 in 0.1% DMSO. Points with the same letters at the top were not significantly different (p > 0.05) according to one-way ANOVA test.
neutral, electron-hole separation and greater catalytic reactivity (Bickley, 1991). Therefore, most alkaline, and acidic solutions can be illustrated by the following reactions.

In neutral and acidic solution (Fujishima and Honda, 1972):

\[ \text{H}_2\text{O} + h^+ \rightarrow \cdot\text{OH} + \text{H}^+ \] (2.2)

In alkaline solution (Sato and White, 1980):

\[ \text{OH}^- + h^+ \rightarrow \cdot\text{OH} \] (2.3)

Under alkaline conditions, hydroxyl radicals occupied most of the ROS (Lair et al., 2008), as indicated by the results of the current study. Hydroxyl radicals are more readily generated at higher pH (Zheng et al., 1997) which may explain why photocatalytic degradation of BDE-209 and production of lower congeners were the most vigorous at pH 12.

2.3.3.2 At Different Concentrations of Humic Acid

The present study showed that 20 mg/L of humic acid had the highest RFU ratio of treatments over the controls (2.68 ± 0.0907) (Fig. 2.3b). This was an uprising trend from 0 mg/L but it dropped at 40 mg/L of humic acid (1.64 ± 0.0616). A similar observation was found for BDE-47 (23.6 ± 2.20, 30.9 ± 3.31 nmol/L) with peaks presented at 10 and 20 mg/L of humic acid. BDE-99 production (36.8 ± 2.97 nmol/L) was only significantly higher than the control at
20 mg/L of humic acid (p < 0.05), while BDE-209 reduction peaked at 5, 10 and 20 mg/L of humic acid (93.0% ± 1.70%, 91.6% ± 3.21%, 91.9% ± 0.952%, respectively) which were all significantly (p < 0.05) higher than the controls (Fig. 2.5). There was a significant correlation between ROS induction and production of BDE-47 (r = 0.703, p < 0.01) and BDE-99 (r = 0.696, p < 0.01), which illustrated that the increase of ROS may be related to the production of these congeners.

Dissolved organic matter has been proven to promote the photodegradation of organic pollutants such as carboxin and oxycarboxin in aqueous condition (Aguer et al., 2002; Hustert et al., 1999). This may be the result of photosensitization which extends the response of TiO₂ (Zhao et al., 2004). Humic acid was added as photosensitizer in order to extend the absorption spectra into the visible light region (Vinodgopal, 1994). The HA molecules were excited by absorbing visible light and donated electrons to conduction band (CB) of TiO₂ (Cho and Choi, 2002). A photosensitizer can transfer absorbed energy to a chemical or generate oxygen reactive species to enhance the breakdown of organic pollutants (Takahashi et al., 1988). The sensitized reduction process could be subsequently propagated through the oxidation of HA. Humic acid has been used as a photosensitizer in photodegradation of DDTs in the presence of TiO₂.
Figure 2.5 Photocatalytical degradation by TiO$_2$ in 0.1 % DMSO with different concentrations of humic acid. (a) Concentration of BDE-47 and (b) BDE-99, and (c) reduction percentage of BDE-209. Points with the same letter at the top were not significantly different (p > 0.05) according to one-way ANOVA test.
This may be due to the ability of production of ROS by humic acids, such as hydrogen peroxide, hydroxyl radicals and singlet oxygen (Aguer et al., 1999; Sandvik et al., 2000). In addition, the detection of hydroxyl radicals and BDE-209 reduction increased with the concentration of humic acid until 20 mg/L, this suggested that the photosensitization effect of humic acid on TiO$_2$ may be concentration dependent.

2.3.3.3 In Different Crystalline Structures of TiO$_2$

TiO$_2$ can exist naturally as several crystalline forms, including anatase, rutile and mixture of anatase and rutile (Tsuji et al., 2006). The influence of different crystalline forms of the catalyst on ROS production and degradation of BDE-209 were investigated. Results showed that the mixture of anatase and rutile produced the most hydroxyl radicals (RFU ratio = 4.21 ± 0.528) (Fig. 2.3c). The mixture also generated the highest amount of BDE-47 (12.0 ± 1.81 nmol/L) among the three crystalline forms (Fig. 2.6). Significant reduction of BDE-209 compared with control could only be observed in the anatase/rutile mixture (82% ± 3%). A significant negative correlation ($r = 0.696$, $p < 0.05$) obtained between ROS induction and concentration of BDE-209, suggesting that the increase of ROS may be related to BDE-209 degradation. In addition, there were also significant positive correlations between ROS induction and concentrations of BDE-47 ($r = \ldots$)
Figure 2.6 Photocatalytic degradation by different crystalline forms of TiO$_2$ in 0.1 % DMSO. (a) Concentration of BDE-47 and (b) BDE-99, and (c) reduction percentage of BDE-209. Points with the same letter at the top were not significantly different (p > 0.05) according to one-way ANOVA test.
0.819, p < 0.01) and BDE-99 (r = 0.809, p < 0.01), revealing the production of these congeners may be due to the increase of ROS. The photocatalytical function of TiO$_2$ depends on the size and crystalline forms of the particles. When anatase exists together with rutile, anatase may act as an oxidation centre while rutile may act as a reduction centre. Rutile can accept electrons from anatase. The electrons accumulated in rutile can be readily removed by reduction reactions with water or protons (Hongo and Nogami, 2007). A series of ROS species will thus be generated and propagate the photocatalytic degradation.

2.4 Conclusions

There is an urgent need to promote the removal efficiency of PBDEs owing to the rapid production and application in industry. TiO$_2$, a promising photocatalyst used in AOP was applied for the removal of BDE-209. The fact that the optimal conditions for photocatalytical degradation of BDE-209 in 0.1% DMSO which has not been previously reported, accentuated the removal efficiency of PBDEs in advanced sewage treatment. The present study showed that TiO$_2$ can promote the degradation of BDE-209, with a shorter half-life (3.05 days) than natural degradation. The degradability of BDE-209 was attributed to the photocatalytic activity of TiO$_2$ but not the small size of the particles. The photocatalytic degradation of BDE-209 performed best at pH 12 (93% ± 1%), 5,
10, 20 mg/L (93.0% ± 1.70%, 91.6% ± 3.21%; 91.9% ± 0.952%), respectively of humic acid and in the form of anatase/rutile TiO$_2$ (82% ± 3%). Collectively, the findings provide crucial information about the optimal conditions for the photocatalytical degradation of PBDEs and may also improve the advanced oxidation process in wastewater treatment facilities before applying further treatment. Hence, the removal efficiency of PBDEs can be enhanced and operation cost may be reduced.
CHAPTER 3
UPTAKE, TRANSLOCATION AND TRANSPORT MECHANISMS OF BDE-209 BY RICE (ORYZA SATIVA) ASSOCIATED WITH ARBUSTULAR MYCORRHIZAL FUNGI

3.1 Introduction

Among the 209 congeners, deca-BDEs contribute to the majority of production and consumption of PBDEs (30000 tonnes/year), which is about 75% (Sjödin et al., 1999). The release of PBDEs into the environment can be attributed to the synthesis process, inappropriate disposal, incorporation into products, and from hazardous waste sites (Wang, 2007). For example, BDE-209 was the dominant congener of the PBDEs (2720–4250 ng/g, dry w) detected in soils from an acid leaching site (using acids to recover metals from electronic boards) of Guiyu, the world's mega site for e-waste recycling (Leung et al., 2007).

Phytoremediation, which is a natural, environmentally friendly and socially acceptable technology, is commonly employed for remediation of contaminated soils (Alkorta and Garbisu, 2001). The uptake of PBDEs from soils by plants has been investigated (Huang et al., 2010; Huang et al., 2011; Wang et al., 2011). In
general, PBDEs were accumulated in all tested plant species, with the concentrations in roots higher than that in shoots. A higher proportion of lower brominated congeners was observed in the plant tissues than those in the soil samples, implying that they might be more readily taken up by the plants or further metabolism of PBDEs within the plants might occur (Huang et al., 2010). In order to facilitate phytoremediation, arbuscular mycorrhizal fungi (AMF) (the most common mycorrhizal type), which are ubiquitous symbionts, have been applied into the rhizosphere of the host plants (e.g. ryegrass) for degrading some organic pollutants (e.g. PAHs) (Smith and Read, 2008; Yu et al., 2011). Nevertheless, there is still a lack of thorough study in the phytoremediation of PBDEs with inoculation of AM fungi. To the best of our knowledge, the only related study was conducted by Wang et al., 2011, which has only investigated one species of AMF in the Glomus genus (Glomus mosseae (GM)) associated with Italian ryegrass (Lolium multiflorum L.). Moreover, the effects of environmental changes (e.g. pH levels of soil) on the uptake of PBDEs by plants remain unknown. Although it has been proven that plant lipid was associated with the sorption of PBDEs by root (pumpkin, maize or ryegrass), the sorption mechanism of PBDEs into plant roots is largely unknown (Huang et al., 2011). In addition, the roles of cells in the uptake of PBDEs by plant are not clear. Therefore, there is an
urgent need to understand the uptake mechanisms of BDE-209 in plants in order to provide more comprehensive information for the phytoremediation of soil contaminated with BDE-209.

A partition-limited model was proposed to deal with the transport of non-ionic organic contaminants from soil to plants (Chiou et al., 2001). Through the application of the model, the concentration of a contaminant at particular part of a selective plant at a given time can be determined by the concentrations in soil (Chiou et al., 2001). Furthermore, the model also describes the extent of approach to equilibrium, which is indicated by the “quasi-equilibrium factor \( \alpha_{pt} \)” derived from concentrations of the contaminant in plant parts and soil, specific partition coefficients and weight fractions of the plants (Chiou et al., 2001). The model has been applied in various studies concerning plant uptake of organic contaminants ((PAHs and ryegrass): Gao et al., 2005; (PAHs and ryegrass): Yang and Zhu, 2007). In general, most of the calculated \( \alpha_{pt} \) values in water-plant or soil-plant systems of the studied hydrophobic organic contaminants (e.g. phenanthrene and pyrene) were smaller than 1, referring to non-equilibrium state of the system (Gao et al., 2005).

In addition, attention should also be paid on the role of cellular activities in the uptake of organic contaminants by plant roots. Tames and Hance (1969)
investigated the uptake of five herbicides (atrazine, diuron, linuron, monolinuron and GS 14260) by the freshly killed roots of oat, bean, pea, cucumber and radish, and revealed that only the external root surface may involve in the adsorption of herbicides. In the in situ visualization study of anthracene and phenanthrene by two-photon excitation microscopy (TPEM) techniques, dominated apoplastic movement of the PAHs compounds through cell walls was observed (Wild et al., 2005).

The present study aimed to investigate the uptake behavior and mechanisms of BDE-209 in soil-plant system involving rice plant. Rice (Oryza sativa) was chosen as a model plant because it was the first crop with a complete genome sequence (International Rice Genome Sequencing Project, 2005). Furthermore, its ability in the uptake of six organic compounds, dinitrobenzene, dinitrotoluene, lindan, 1,2,3-triclorobenzene, phenanthrene and pyrene is known (Su and Zhu, 2007). Based on the background information, it was hypothesized that degradation of BDE-209 in sand may be enhanced by planting with rice plants (Fengmeizhan, Hefengzhan and Guangyinzhan). The objectives of the current study included (1) uptake and translocation of BDE-209 by three rice cultivars namely Fengmeizhan, Hefengzhan and Guangyinzhan, (2) effects of changing soil pH levels on the uptake of BDE-209 by rice, (3) influences of colonization of AM fungi in rice
roots on BDE-209 dissipation in soil, and (4) uptake mechanisms of BDE-209 into rice roots.

3.2 Materials and methods

3.2.1 Sand Preparation

Sand, purchased from a local gardening store in Hong Kong, without any detectable PBDEs was employed in this study. It was washed with deionised water and oven dried at 75 °C. It was then sieved through 2-mm sieve and autoclaved at 121 °C for 2 h to eliminate the indigenous micro-organisms. BDE-209 (Dr. Ehrenstorfer (Germany)) was spiked into the sand according to the method described by Mueller et al. (2006) and Wang et al. (2011). A small batch of sand (1 kg, approximately 10% of the final amount) was spiked with a solution of BDE-209 (750 µg) dissolved in 100 mL in acetone. The spiked sand was then tumbled with unspiked sand for 2 h at room temperature (25 °C). The mixed sand was then allowed for complete evaporation of solvent in dark in the fume hood and shaken for 30 min for homogenization every day in an autoclaved bottle in dark. The sand was then flushed with 0.5 mM phosphate-buffered solution (PBS) adjusted to pH 6, 7 and 8, respectively, for 24 hours (Van Aarle et al., 2002). The mixing finally resulted in a concentration of 79.3 nmol/kg for total BDE-209 (Mueller et al., 2006; Wang et al., 2011).
3.2.2 Inoculation of AM Fungi in Sand

Three species of AM fungi (Glomus intraradices (GI), Glomus mosseae (GM) and Glomus versiforme (GV)) were selected for the current study due to their prominent effects on the promotion of biomass, phosphate concentration, and colonization rate of rice plants (Prakash) (Secilia and Bagyaraj, 1992). Furthermore, Glomus intraradices and Glomus mosseae have been reported to help in the tolerance and rhizodegradation of PAHs and BDE-209, respectively (Verdin et al., 2006; Wang et al., 2011). Four mycorrhizal treatments included the control (without AM fungi) and three species of AM fungi (G. intraradices was obtained from MycAgro Lab., France and the other two AMF were obtained from Bank of Glomales in China (BGC), Beijing Academy of Agriculture and Forestry Sciences, China) were set up. In each pot of mycorrhizal treatments, 40 g of AMF inoculum was added into 1.2 kg sand (BDE-209 spiked or non-BDE-209 spiked) (Li et al., 2011).

3.2.3 Pot Experiments with Rice Plants

Three lowland rice (Oryza sativa L.) seeds (Fengmeizhan, Guangyinzhan and Hefengzhan), commonly grown in southern China, were obtained from Guangdong Academy of Agricultural Sciences, China. They were surface sterilized in a 30% H$_2$O$_2$ (wt/wt) solution for 10 min, followed by thoroughly rinsing with
sterilized deionized water. The sterilized seeds were then placed on sterilized nylon mesh for germination in dark in a growth chamber (Sanyo MRL-351H, Japan). The seedlings were transferred to 20% Hoagland’s nutrient solution (1.0 mM Ca(NO$_3$)$_2$, 1.0 mM KNO$_3$, 0.4 mM MgSO$_4$, 0.2 mM KH$_2$PO$_4$, 10 μM Fe(II)-EDTA, 9 μM H$_3$BO$_4$, 0.2 μM ZnSO$_4$, 0.1 μM CuSO$_4$, 2 μM MnSO$_4$, 0.02 μM (NH$_4$)$_6$Mo$_7$O$_{24}$) (Hoagland and Arnon, 1938) at day 7 after germination. Seedlings with similar size (around 15 cm in length) were selected for the pot experiments. Non-spiked sand with plant and spiked sand without plant (sowed as controls) were set up. Five rice seedlings were transplanted in every plant treatment pot. The upper top soil 5-6 cm of each pot was covered with non-spiked sterilized soil to establish a buffer layer to reduce the loss of BDE-209 due to evaporation and photolysis (Wang et al., 2011). The positions of the pots (placed in a greenhouse at Hong Kong Baptist University with temperature control, 28/23 °C day/night) were re-randomized every 3 days and all the treatments were conducted in triplicate. All the pots were watered with deionized water every day and conditioned with 20% Hoagland’s nutrient solution (Hoagland and Arnon, 1938) mixed with PBS buffer adjusted to pH 5 or 9 once a week in order to maintain the pH levels of the pots.

3.2.4 Harvesting and Sample Preparation

After 60 days, plant shoots and roots were harvested separately, with roots
carefully removed from the soil. Shoot and root samples were rinsed three times with D.I. water separately, blotted with tissue papers. The BDE-209 free sand on the top was removed and the sand in the rhizospheric soil was collected. A portion of the freshly collected rice roots (about 2 g) was reserved for AMF colonization rate analysis. The rest of the plant and sand samples were frozen at –20 °C overnight, and then freeze-dried for 3 to 7 days. After weighing the dry weight, the dried shoot and root samples were ground separately and stored at –20 °C before chemical analysis (US EPA, 2007).

3.2.5 Quantification of Root Colonization

The freshly collected root samples were washed with D.I. water for 3 times to remove adhered sand and then put into 10% (w/v) potassium hydroxide (KOH) solution. The solution containing the roots was heated at 90 °C for 40 min. The KOH solution was then discarded and the roots were washed with D.I. water for 3 times. The root tissues were then bleached with fresh alkaline H₂O₂ solution for 15 min (30 mL 10% H₂O₂ + 3 ml of NH₄OH + 567 mL D.I. water). The root tissues were again washed three times with D.I. water. The samples were acidified by immersing in 1% hydrochloric acid (HCl) for 3-4 min, followed by staining with 0.05% lactophenol blue in 90 °C water bath for 15 min. The roots were then destained by immersing in deionized (D.I.) water and finally examined for fungal
colonization under microscope (Philips and Hayman, 1970). A drop of glycerol and lactic acid solution (1:1 = v:v) was added on twenty segments of stained roots (each approximately 1 cm long) placed on a glass slide and then covered with a cover slip. There were three replicates for each subsample. The length of infected cortex was recorded under microscopic assessment and calculated as a percentage of colonization (Giovannetti and Mosse, 1980).

3.2.6 Lipid Extraction

Total lipid in roots was extracted by mixing chloroform–methanol (1:2 v/v) with the samples according to the method in Bligh and Dyer (1959). The lipid fraction was recovered from the solvent mixture after shaking for 5 min and the solvent mixture with extracted lipid was concentrated using a rotary evaporator. The weight of the extracted lipid of each sample was measured for the calculation of total root lipid content (Lee et al., 2010).

3.2.7 Dissolved Organic Carbon (DOC) in Sand

The DOC of fresh soil samples was extracted with deionized water (soil: water = 1:5). The extract was then analyzed with a total organic carbon (TOC) analyzer (Shimadzu TOC-Vcph, Japan).

3.2.8 Partition-limited Model

A partition-limited model was applied for describing and estimating the
uptake mechanisms of BDE-209 by rice roots. When the quasi-equilibrium factor ($\alpha_{pt}$) is equal to 1, an equilibrium state is reached. A passive transport dominated process should be signified by a $\alpha_{pt}$ value smaller than 1 (Chiou et al., 2001). In the typical cases of soil contamination, the uptake of organic contaminants in soil was derived from equation 3.1.

$$C_{pt} = \alpha_{pt} (C_{som}/K_{som})[f_{pom} K_{pom} + f_{pw}]$$

(3.1)

where $C_{pt}$ is the concentration of contaminant in a specific part of the plant, while $f_{pom}$ and $f_{pw}$ are the total weight fraction of the organic matter and water in the plant respectively, $K_{pom}$ is the partition coefficient between plant organic matter and plant water (Chiou et al., 2001). Octanol is usually assimilated to biological lipids, so $K_{pom}$ are assumed to be the same as the $K_{ow}$ of PBDEs (Chiou et al., 2001). It was also assumed that metabolism does not affect the passive transport of BDE-209 (Chiou et al., 2001). Soil organic matter may alter the bioavailability of a contaminant in soil (Cunningham and Ow, 1996), and therefore the concentration in soil should be normalized with the SOM content in the soil in equation 2, i.e. SOM-normalized contaminant concentration in soil ($C_{som}$) which can be expressed as the following:

$$C_{som} = C\cdot f_{som}$$

(3.2)

where $f_{som}$ is the weight fraction of soil organic matter in soil (Chiou et al., 2001).
$K_{\text{som}}$ in equation 1 is the contaminant partition coefficient between SOM and water, and can be derived from $C_w$ and $C_{\text{som}}$ in the following equation (Chiou et al., 2001).

$$C_w = C_{\text{som}}/K_{\text{som}}$$

(3.3)

where $C_w$ can be obtained from the relation of the distribution coefficient between soil and water for a specific contaminant in a particular soil ($K_d$) and the contaminant in the soil ($C_s$) in the following equation (Chiou et al., 2001).

$$C_s = K_d C_w$$

(3.4)

3.2.9 Preparation of Rice Roots for Sorption Analysis

Rice (*Oryza sativa* L.) seedings (Fengmeizhan, Guangyinzhan and Hefengzhan) were prepared as mentioned in Section 3.2.3. The rice roots were then rinsed three times with deionised water and subsequently dried with tissue papers. The roots were cut at the basal node and the fresh weight of the root segments was about 1.0 g. Part of the roots was boiled at 105 °C for 40 min (Su and Zhu, 2007).

3.2.10 Uptake Isotherms of BDE-209 with Fresh and Dead Roots

The fresh and dead roots were rinsed with sterilized deionized water and dried with clean tissue papers. The roots were then added into 30 mL 0.01 M CaCl$_2$ solutions spiked with particular concentration of BDE-209. BDE-209 was previously dissolved into acetone before spiking into the solution. The glass
bottles were then sealed and shaken at 25 °C for 16 h. BDE-209 solution with no rice roots and solution with 0.01 M CaC\textsubscript{12} only were used as controls. All the treatments were in triplicates and prevented from photodegradation of BDE-209 by wrapping the glass bottles with aluminium foil. The concentrations of BDE-209 in the fresh and dead roots and solutions were determined separately and an uptake isotherm was subsequently constructed (Su and Zhu, 2007).

3.2.11 Extraction of PBDEs

The extractions of PBDEs in plant and sand were based on Standard Methods 1614 and 3540C respectively (US EPA, 2007, US EPA, 1996b). Ten grams of sand sample or two to five grams of plant tissue were spiked with 100 µL of 200 ppb mass-labelled (\textsuperscript{13}C\textsubscript{12}) solution/mixture (MBDE-MXE) (Wellington Laboratories Inc, Canada) for extraction recoveries of PBDEs. The samples were placed in a thimble added with 5 g anhydrous sodium sulphate (US EPA, 2007). The samples were then extracted with 90 mL acetone (pesticide grade, Tedia), dichloromethane (DCM) (pesticide grade, Tedia) and n-Hexane mixture (1:1:1, v:v:v) in a 150-mL round-bottomed flask connected with Soxhlet apparatus for 18 h (US EPA, 1996b). The extract was allowed to cool after the extraction was completed. The extract was then concentrated to around 10 mL by a rotary evaporator, followed by cleaning up using a standard clean-up method 3620B (US
EPA, 1996c). In short, extracts were eluted with n-hexane (pesticide grade, Tedia) in a florisil packed glass column (US EPA, 1996c). After the clean-up, the extracts were reduced to around 1 mL by a rotary evaporator and evaporated by a gentle stream of nitrogen gas until the volume reached 100 μL. Then, $^{13}$C$_{12}$-BDE-138 (200 ng/mL) was added to the sample before GC injection as the injection standard.

3.2.12 Chemical Analysis of PBDEs

GC-MS analysis was carried out on Agilent 7890A GC-MS instrument connected with an Agilent 5975C inert Mass Selective Detector triple-axis detector (Agilent Technologies, USA) and a 15 m x 0.25 mm x 0.25 μm capillary column (Agilent technologies). Concentrations of 30 PBDEs (3, 7, 15, 17, 28, 47, 49, 66, 71, 77, 85, 99, 100, 119, 126, 138, 153, 154, 156, 183, 184, 191, 196, 197, 206, 207, 209) were determined based on the Standard Method 1614 (US EPA, 2007). The temperature program of the GC oven was: temperature held at 100 °C for 5 min, increased at 10 °C/min to 310 °C, and then held for 20 min. A 1-μL of each sample was injected to the GC column.

3.2.13 Quality Control

Extraction and analysis were conducted in dark, in order to minimize the exposure to light. A procedure blank was included in each batch of extraction. The
LOD which was defined as three times the standard deviation in the blank samples, ranged from 0.5 ng/g (BDE-85) to 4 ng/g (BDE-206). The LOD of BDE-209 was 95 ng/g. A mass-labelled polybrominated diphenyl ethers \( ^{13} \text{C}_{12}-\text{PBDEs} \) (PBDEs 3, 15, 28, 47, 99, 153, 154, 183, 197, 207 and 209) (40 ng/mL) were added into samples prior to extraction and the recoveries ranged from 70.2-105.5%.

3.2.14 Data Analysis

The statistical analyses of the data were performed by using the SPSS version 16 software package for Microsoft Windows. The statistical values were all calculated for triplicates. Normality of the data was checked by a Shapiro-Wilk test before all further statistical calculations. A 95% confidence limit (p < 0.05) was applied for the indication of significant differences between samples.

3.3 Results and Discussion

3.3.1 BDE-209 Dissipation and Debromination in Sand Planted with *Oryza sativa* (Rice)

The residual concentrations of BDE-209 in sand after 60-day cultivation of three different cultivars of rice at different pHs are shown in Fig. 3.1a. Significant differences (p < 0.05) were observed in the concentrations of BDE-209 in sand with various pH levels (Fig. 3.1a). In all cultivars, the concentrations of BDE-209
Figure 3.1 Dissipation and accumulation of BDE-209 in sand and plant tissues (roots and shoots) at different pHs. (a) Concentrations of BDE-209 in sand, (b) in roots and (c) in shoots of rice. Bars with the same letter at the top were not significantly different (p > 0.05) according to one-way ANOVA test.
were the lowest at pH 7 (e.g. 33.1 nmol/kg in Hefengzhan) but highest at pH 8 (e.g. 46.3 nmol/kg in Hefengzhan), indicating the dissipation of BDE-209 in sand planted with rice was influenced by the pH levels (Fig. 3.1a). The concentration of BDE-209 in the unplanted control sand was 75.2 nmol/kg on average, with only 5.2% loss of the initial concentrations (79.3 nmol/kg), and with no significant difference in BDE-209 concentrations in sand among various pHs (Fig. 3.1a). Comparing with those planted with rice, the unplanted control sand presented a less prominent dissipation of BDE-209, which was possibly attributed by evaporation or sorption on the containers. The pHs of sand did not exert any significant effect on the dissipation of BDE-209 without plant, but it did play an essential role in dissipation of BDE-209 in sand planted with rice.

Debromination was noted by the detection of 9 to 15 lower brominated PBDE congeners (di- through nona-) in the sand after cultivation of the three rice cultivars (Fig. 3.2a). In all pots, nona-BDEs (BDE-206, -207) and deca-BDE (BDE-209) dominated the congeners profile of the sand and the mean of the total percentages of these two groups ranged from 87.0 to 95.9%. This suggested that BDE-209 inclined to lose one bromine atom to form nona-BDE during metabolism in the rhizosphere of rice, which coincided with the results obtained by Wang et al. (2011), who investigated the effect of root colonization with AM
Figure 3.2 Degradation profile of BDE-209 in sand and plant tissues (roots and shoots) at different pHs. (a) PBDE congeners profile in sand, (b) in roots and (c) in shoots of rice.
fungi (*Glomus mosseae*) on rhizospheric degradation of BDE-209 in soil. Results showed that the various pH levels (pH 6-8) did not lead to apparent variation in PBDE congeners profile, except a slight difference between the proportions of BDE-209. Since the sand was sterilized in an autoclave at 121 °C for 2 h prior to spiking of BDE-209, microbial debromination of BDE-209 by indigenous microbes was prohibited. Consequently, adsorption, translocation and metabolism by rice were possibly the major causes of the degradation of BDE-209 in sand.

3.3.2 Uptake and Accumulation of BDE-209 in Rice

The concentrations of BDE-209 in roots and shoots of different rice cultivars are illustrated in Fig. 3.1b and c, respectively. The results of BDE-209 accumulation in plant tissues of rice were consistent with those in sand. The greatest amounts of BDE-209 accumulated in roots and shoots at pH 7 were observed in Hefengzhan (19.2 and 0.36 nmol/kg, respectively). However, no significant difference was observed in BDE-209 concentrations in the plant tissues (both roots and shoots) of Fengmeizhan among the three pHs, implying that changes of pH did not pose any effect on the uptake and accumulation of BDE-209 in this cultivar. However, an apparent drop of BDE-209 appeared at pH 7 in sand, indicating part of BDE-209 was degraded by certain pathways (e.g. plant-derived degradative enzymes) other than adsorption and uptake by plants.
Plant-derived enzymes in root exudation, such as laccases, peroxidases, dehalogenases, nitroreductases and nitrilases, may help to degrade the organic contaminants in soil (Chroma et al., 2002). Peroxidases have been reported to be able to metabolise PCBs which is structurally similar to PBDEs (Chroma et al., 2002). In addition, quantitative or qualitative changes in root exudation could vary sorption of PBDEs to soil particles and subsequently influence the degradation of PBDEs (Mueller et al., 2006). Significant differences (p < 0.05) in the accumulation of BDE-209 were observed among the three cultivars at pH 7 in the order of Hefengzhan > Guangyinzhan > Fengmeizhan (ranging from 16.3 to 19.6 nmol/L). The higher levels of BDE-209 recorded in both roots and shoots of Hefengzhan than the two other cultivars at pH 7 (p < 0.05) demonstrated the variation in abilities of the cultivars in the uptake and accumulation of BDE-209 at pH 7.

According to the PBDE congeners profile, BDE-209 was detected in both roots and shoots, elucidating the ability of rice to take up and accumulate BDE-209 in plant tissues (Fig. 3.2b and c). On the other hand, there were more lower brominated PBDE congeners detected in the plant tissues than those in sand, indicating the translocation and further metabolism of PBDEs in the soil-plant systems (Fig. 3.2b and c). The results are in line with those of previous studies.
involving other plants, including Italian ryegrass, alfalfa, pumpkin, summer squash and maize (Huang et al., 2010; Huang et al., 2011).

The present study observed a significant positive correlation \( (r = 0.684, p < 0.05) \) between the concentrations of BDE-209 in roots and root lipid contents. It has been proposed that plant lipid content may play an essential role in the uptake of organic contaminants, such as PAHs and PBDEs (Su and Zhu, 2007; Huang et al., 2010). Huang et al. (2010) also specified that the accumulation of BDE-209 in roots was related to root lipid contents.

3.3.3 Contribution of AMF to Growth of Rice in BDE-209 Contaminated Sand

Table 3.1 illustrates the mycorrhizal infection rates of rice after 60-day cultivation. The colonization rate of the AMF inoculated rice roots varied among AMF species and pHs, while the non-inoculated rice roots control showed no colonization (Table 3.1). Generally, there was no significant difference in root colonization rates between BDE-209 spiked and non-spiked treatments \( (p > 0.05) \), implying that addition of BDE-209 into the sand did not lead to any observable alteration of the colonization rates of AMF in rice roots (Table 3.1). There was also no significant difference in root colonization rates among different rice cultivars \( (p > 0.05) \) (Table 3.1). In most of the treatments, changes in pHs did not
Table 3.1 Mycorrhizal infection rates of rice roots (% of total root length infected) at different pHs.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Fengmeizhan (ctrl)</th>
<th>Hefengzhan (ctrl)</th>
<th>Guangyinzhan (ctrl)</th>
<th>Fengmeizhan (BDE-209)</th>
<th>Hefengzhan (BDE-209)</th>
<th>Guangyinzhan (BDE-209)</th>
</tr>
</thead>
<tbody>
<tr>
<td>pH 6 (GI)</td>
<td>16.2 Aa</td>
<td>16.6 Aa</td>
<td>16.2 Aa</td>
<td>16.0 Aa</td>
<td>16.1 Aa</td>
<td>16.4 Aa</td>
</tr>
<tr>
<td>pH 6 (GM)</td>
<td>12.2 Ab</td>
<td>11.6 Ab</td>
<td>12.2 Ab</td>
<td>12.5 Aa</td>
<td>11.5 Ab</td>
<td>11.9 Ab</td>
</tr>
<tr>
<td>pH 6 (GV)</td>
<td>2.75 Ac</td>
<td>2.56 Ac</td>
<td>2.69 Ac</td>
<td>2.60 Ab</td>
<td>2.66 Ac</td>
<td>2.70 Ac</td>
</tr>
<tr>
<td>pH 7 (GI)</td>
<td>16.9 Aa</td>
<td>17.3 Aa</td>
<td>16.6 Aa</td>
<td>16.3 Aa</td>
<td>16.2 Aa</td>
<td>16.3 Aa</td>
</tr>
<tr>
<td>pH 7 (GM)</td>
<td>12.2 Ab</td>
<td>12.3 Ab</td>
<td>11.3 Ab</td>
<td>13.3 Ab</td>
<td>12.6 Ab</td>
<td>11.4 Ab</td>
</tr>
<tr>
<td>pH 7 (GV)</td>
<td>2.80 Ac</td>
<td>2.50 Ac</td>
<td>2.72 Ac</td>
<td>2.58 Ac</td>
<td>2.47 Ac</td>
<td>2.73 Ac</td>
</tr>
<tr>
<td>pH 8 (GI)</td>
<td>13.0 Aa</td>
<td>13.8 Aa</td>
<td>13.7 Aa</td>
<td>13.6 Aa</td>
<td>13.3 Aa</td>
<td>13.5 Aa</td>
</tr>
<tr>
<td>pH 8 (GM)</td>
<td>12.0 Aa</td>
<td>11.6 Ab</td>
<td>11.9 Aa</td>
<td>12.5 Aa</td>
<td>12.2 Aa</td>
<td>11.5 Aa</td>
</tr>
<tr>
<td>pH 8 (GV)</td>
<td>2.55 Ab</td>
<td>2.57 Ac</td>
<td>2.66 Ab</td>
<td>2.67 Ab</td>
<td>2.67 Ab</td>
<td>1.20 Bb*</td>
</tr>
</tbody>
</table>

Ctrl refers to non-spiking control of BDE-209 treatment; GI refers to *Glomus intraradices*; GM refers to *Glomus mosseae*; GV refers to *Glomus versiforme*. Values with the same small letter are not significantly different among different AMF species in the same treatment. Values with the same capital letter are not significantly different among pHs in the same BDE-209 treatment. Values with asterisk (*) are significantly different from the control according to independent T-test ($p < 0.05$).
exert observable effects on the root colonization rate (Table 3.1). Significant variations in root colonization rates among pHs were only observed in GI inoculated rice roots (p < 0.05), in which the lowest colonization rate was found at pH 8 (e.g. Hefengzhan (GI) at pH 8 (ctrl) = 13.8%). This result was in line with those obtained by Van Aarle et al. (2002), which observed a decreased total AM root colonization (79%) by *G. intraradices* with lower arbuscule (40%) and vesicle formation (16%) at a relatively higher pH (pH 6). Among the three AMF species (GI, GM and GV), GI presented the highest root colonization rates in rice roots (e.g. Hefengzhan (ctrl) at pH 7 (GI) = 17.3%). The three tested AMF species, in descending order of mycorrhizal root colonization rates were GI > GM > GV in this study (Table 3.1). This result coincided with that in Secilia and Bagyaraj (1992), which stated GI had the greatest infectious ability to rice roots among the three AMF species.

Inoculation of rice with AMF significantly promoted the biomass of roots and shoots when compared with non-inoculated control (Table 3.2 and 3.3) (p < 0.05). The benefits of AMF in plant growth have already been widely investigated. The biomass enhancement was attributed to increased phosphate uptake (Secilia and Bagyaraj, 1992), while the phosphate uptake was closely related to the rice phosphate transporter gene OsPT11 (Paszkowski et al., 2002). This gene was
specifically activated by arbuscular mycorrhizal symbiosis (Paszkowski et al., 2002). The greatest biomass of root and shoot (in dry weight basis) was associated mostly in the treatment inoculated with GI (e.g. root of Hefengzhan (ctrl) at pH 7 (GI) = 7.57 g). This result agreed with those from Secilia and Bagyaraj (1992), in which GI showed the best performance in enhancing biomass and mycorrhizal root colonization rates. Comparing with non-spiked control, spiking of BDE-209 did not exert a prominent difference in the biomass of roots and shoots (p > 0.05), indicating that addition of BDE-209 into the sand did not result in changes of plant biomass under the inference of AMF (Table 3.2 and 3.3). In both biomass of roots and shoots, Hefengzhan generally showed a significantly higher value than the other two cultivars in non-inoculated control or AMF inoculated treatment (p < 0.05) (e.g. root of Hefengzhan (ctrl) at pH 7 (NAMF) = 5.43 g). The majority of biomass of rice (roots and shoots) was not significantly varied between different pHs (p > 0.05), but the biomass (of root and shoot) was found significantly lower at pH 8 than the other two pHs in some treatments (p < 0.05) (e.g. shoot of Hefengzhan (GM) at pH 8 (ctrl) = 8.26 g). Significant correlations were found between root colonization rates and root biomass of all studied rice cultivars (Fengmeizhan: $r = 0.525$, p < 0.01; Hefengzhan: $r = 0.589$, p < 0.01; Guangyinzhan: $r = 0.575$, p < 0.01). These suggested that the root biomass of rice
Table 3.2 Biomass of rice roots of different rice cultivars under the influence of AMF.

<table>
<thead>
<tr>
<th>Treatments</th>
<th>Fengmeizhan (ctrl)</th>
<th>Hefengzhan (ctrl)</th>
<th>Guangyinzhan (ctrl)</th>
<th>Fengmeizhan (BDE-209)</th>
<th>Hefengzhan (BDE-209)</th>
<th>Guangyinzhan (BDE-209)</th>
</tr>
</thead>
<tbody>
<tr>
<td>pH 6 (NAMF)</td>
<td>3.09 Bc</td>
<td>4.37 Ac</td>
<td>3.10 Bc</td>
<td>3.50 Ab</td>
<td>4.56 Aa</td>
<td>3.46 Aa</td>
</tr>
<tr>
<td>pH 6 (GI)</td>
<td>5.40 ABa</td>
<td>6.44 Aa</td>
<td>5.07 Ba</td>
<td>4.66 Aa</td>
<td>5.90 Aa</td>
<td>4.75 Aa</td>
</tr>
<tr>
<td>pH 6 (GM)</td>
<td>4.34 Bb</td>
<td>5.60 Aab</td>
<td>4.30 Bb</td>
<td>3.68 Aab</td>
<td>5.22 Aa</td>
<td>4.01 Aa</td>
</tr>
<tr>
<td>pH 6 (GV)</td>
<td>4.12 Bb</td>
<td>5.44 Ab</td>
<td>3.74 Bbc</td>
<td>4.00 Bab</td>
<td>5.45 Aa</td>
<td>4.13 Ba</td>
</tr>
<tr>
<td>pH 7 (NAMF)</td>
<td>4.37 Bb</td>
<td>5.43 Ab</td>
<td>4.03 Ba</td>
<td>3.21 Bb</td>
<td>5.33 Ab</td>
<td>3.50 Ab</td>
</tr>
<tr>
<td>pH 7 (GI)</td>
<td>6.03 ABA</td>
<td>7.57 Aa</td>
<td>5.34 Ba</td>
<td>6.38 Ba</td>
<td>7.76 Aa</td>
<td>6.18 Ba</td>
</tr>
<tr>
<td>pH 7 (GM)</td>
<td>4.40 Ab</td>
<td>5.76 Aa</td>
<td>4.33 Aa</td>
<td>3.35 Bb</td>
<td>5.66 Ab</td>
<td>4.04 Bb</td>
</tr>
<tr>
<td>pH 7 (GV)</td>
<td>4.00 Ab</td>
<td>5.43 Ab</td>
<td>4.08 Aa</td>
<td>4.22 Bb</td>
<td>5.45 Ab</td>
<td>3.81 Bb</td>
</tr>
<tr>
<td>pH 8 (NAMF)</td>
<td>3.31 Ac</td>
<td>4.38 Ab</td>
<td>3.45 Aa</td>
<td>2.97 Aa</td>
<td>4.13 Ab</td>
<td>3.70 Aa</td>
</tr>
<tr>
<td>pH 8 (GI)</td>
<td>5.01 Ba</td>
<td>6.40 Aa</td>
<td>4.64 Ba</td>
<td>4.71 Ba</td>
<td>6.18 Aa</td>
<td>4.64 Ba</td>
</tr>
<tr>
<td>pH 8 (GM)</td>
<td>3.66 Bbc</td>
<td>5.58 Aab</td>
<td>4.18 Ab</td>
<td>4.06 Aa</td>
<td>5.11 Aab</td>
<td>4.01 Aa</td>
</tr>
<tr>
<td>pH 8 (GV)</td>
<td>4.33 Aab</td>
<td>4.91 Aab</td>
<td>4.26 Aa</td>
<td>3.59 Ba</td>
<td>5.29 Aab</td>
<td>3.98 Aa</td>
</tr>
</tbody>
</table>

NAMF refers to the control with no AMF inoculation. GI refers to *Glomus intraradices*; GM refers to *Glomus mosseae*; GV refers to *Glomus versiforme*. Values with the same small letter are not significantly different among different AMF species in the same treatment. Values with the same capital letter are not significantly different in the same BDE-209 treatment.
Table 3.3 Biomass of rice shoots of different rice cultivars under the influence of AMF.

<table>
<thead>
<tr>
<th>Treatments</th>
<th>Fengmeizhan (ctrl)</th>
<th>Hefengzhan (ctrl)</th>
<th>Guangyinzhan (ctrl)</th>
<th>Fengmeizhan (BDE-209)</th>
<th>Hefengzhan (BDE-209)</th>
<th>Guangyinzhan (BDE-209)</th>
</tr>
</thead>
<tbody>
<tr>
<td>pH 6 (NAMF)</td>
<td>5.70 Ab</td>
<td>7.26 Ab</td>
<td>5.94 Ab</td>
<td>5.34 Ab</td>
<td>7.13 Ab</td>
<td>6.29 Aa</td>
</tr>
<tr>
<td>pH 6 (GI)</td>
<td>7.51 Ba</td>
<td>9.97 Aa</td>
<td>7.58 Ba</td>
<td>6.92 Ba</td>
<td>9.48 Aa</td>
<td>6.94 Ba</td>
</tr>
<tr>
<td>pH 6 (GM)</td>
<td>7.27 Ba</td>
<td>10.1 Aa</td>
<td>7.21 Bab</td>
<td>7.03 Ba</td>
<td>10.0 Aa</td>
<td>7.39 Ba</td>
</tr>
<tr>
<td>pH 6 (GV)</td>
<td>6.99 Ba</td>
<td>9.38 Aa</td>
<td>7.12 Bab</td>
<td>6.72 Ba</td>
<td>9.35 Aa</td>
<td>7.06 Ba</td>
</tr>
<tr>
<td>pH 7 (NAMF)</td>
<td>6.52 Bb</td>
<td>8.12 Ac</td>
<td>6.53 Bab</td>
<td>6.61 Bb</td>
<td>7.97 Ac</td>
<td>6.10 Bb</td>
</tr>
<tr>
<td>pH 7 (GI)</td>
<td>8.63 Ba</td>
<td>11.0 Aa</td>
<td>8.67 Ba</td>
<td>8.46 Ba</td>
<td>11.5 Aa</td>
<td>8.65 Ba</td>
</tr>
<tr>
<td>pH 7 (GM)</td>
<td>7.41 Bab</td>
<td>9.98 Ab</td>
<td>7.01 Bb</td>
<td>7.18 Bab</td>
<td>9.97 Ab</td>
<td>6.43 Bb</td>
</tr>
<tr>
<td>pH 7 (GV)</td>
<td>7.04 Bab</td>
<td>9.61 Ab</td>
<td>6.96 Bb</td>
<td>6.97 Bb</td>
<td>9.43 Ab</td>
<td>7.00 Bb</td>
</tr>
<tr>
<td>pH 8 (NAMF)</td>
<td>4.99 Bb</td>
<td>6.95 Ab</td>
<td>5.52 Bb</td>
<td>4.62 Ab</td>
<td>6.53 Ac</td>
<td>5.90 Ab</td>
</tr>
<tr>
<td>pH 8 (GI)</td>
<td>7.52 Ba</td>
<td>10.2 Aa</td>
<td>7.21 Bb</td>
<td>6.98 Ba</td>
<td>9.32 Aab</td>
<td>7.23 Bab</td>
</tr>
<tr>
<td>pH 8 (GM)</td>
<td>8.02 Aa</td>
<td>8.26 Ab</td>
<td>7.26 Aa</td>
<td>7.01 Ba</td>
<td>9.88 Aa</td>
<td>7.87 ABa</td>
</tr>
<tr>
<td>pH 8 (GV)</td>
<td>7.40 Ab</td>
<td>8.97 Ab</td>
<td>6.84 Bab</td>
<td>7.16 Aa</td>
<td>7.64 Aabc</td>
<td>7.09 Aab</td>
</tr>
</tbody>
</table>

NAMF refers to the control with no AMF inoculation. GI refers to *Glomus intraradices*; GM refers to *Glomus mosseae*; GV refers to *Glomus versiforme*. Values with the same small letter are not significantly different among different AMF species in the same treatment. Values with the same capital letter are not significantly different in the same BDE-209 treatment.
plants increased with the AMF colonization rate.

Root lipid content and DOC were reported to be closely linked with the plant uptake of BDE-209 and extractable soil pyrene (Huang et al., 2010; Yu et al., 2011). Therefore, besides biomass, root lipid content and dissolved organic carbon content of sand of rice plants under the interference of AMF were also analyzed. Hefengzhan, which showed the greatest biomass (of roots and shoots), was chosen for further analysis of the influences of pHs, AMF species and BDE-209 spiking on root lipid contents and DOC contents of sand (Tables 3.4 and 3.5). It was found that spiking BDE-209 into the sand did not significantly affect the lipid content of the rice roots and DOC content of sand ($p > 0.05$). However, inoculation of AMF significantly enhanced the root lipid contents and DOC content of sand when compared with the non-inoculated control ($p < 0.05$). In the root lipid content analysis, variation in pH levels was only found in non-inoculated control but not in AMF inoculated treatment. This might reflect pH adaptation of Hefengzhan enabled by the AMF inoculated. In DOC content analysis, the results were quite different from those of biomass and root lipid content. The highest DOC concentration was generally demonstrated in the soil with pH 8 ($p < 0.05$) (e.g. GI at pH 8 (ctrl) = 116 mg/kg). This was possibly due to the increased negative charges on both organic matter and soil inorganic solid surfaces by higher pHs
Table 3.4 Root lipid content of Hefengzhan under the influence of AMF.

<table>
<thead>
<tr>
<th>Treatments</th>
<th>pH 6 (ctrl)</th>
<th>pH 7 (ctrl)</th>
<th>pH 8 (ctrl)</th>
<th>pH 6 (BDE-209)</th>
<th>pH 7 (BDE-209)</th>
<th>pH 8 (BDE-209)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Non AMF</td>
<td>8.03 ABa</td>
<td>8.59 Ab</td>
<td>5.93 Bc</td>
<td>8.65 Ba</td>
<td>11.7 Aab</td>
<td>7.75 Bb</td>
</tr>
<tr>
<td>GI</td>
<td>9.66 Aa</td>
<td>11.7 Aa</td>
<td>11.2 Aa</td>
<td>9.62 Aa</td>
<td>12.3 Aa</td>
<td>11.6 Aa</td>
</tr>
<tr>
<td>GM</td>
<td>8.48 Aa</td>
<td>10.9 Aab</td>
<td>9.56 Aab</td>
<td>8.43 Aa</td>
<td>9.59 Abc</td>
<td>10.1 Aab</td>
</tr>
<tr>
<td>GV</td>
<td>7.77 Aa</td>
<td>8.69 Ab</td>
<td>8.55 Ab</td>
<td>7.43 Aa</td>
<td>8.49 Ac</td>
<td>9.04 Ab</td>
</tr>
</tbody>
</table>

NAMF refers to the control with no AMF inoculation. GI refers to *Glomus intraradices*; GM refers to *Glomus mosseae*; GV refers to *Glomus versiforme*. Values in the same column with the same small letter are not significantly different among different AMF species. Values in the same row with the same capital letter are not significantly different in the same BDE-209 treatment.
Table 3.5 Dissolved organic carbon (DOC) contents of soil planted with Hefengzhan under the influence of AMF.

<table>
<thead>
<tr>
<th>Treatments</th>
<th>pH 6 (ctrl)</th>
<th>pH 7 (ctrl)</th>
<th>pH 8 (ctrl)</th>
<th>pH 6 (BDE-209)</th>
<th>pH 7 (BDE-209)</th>
<th>pH 8 (BDE-209)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Unplanted</td>
<td>28.3 Bd</td>
<td>35.5 ABB</td>
<td>37.5 Ac</td>
<td>29.3 Bc</td>
<td>34.8 ABB</td>
<td>37.3 Ac</td>
</tr>
<tr>
<td>NAMF</td>
<td>56.6 ABc</td>
<td>54.5 Bb</td>
<td>76.2 Ab</td>
<td>53.9 Bb</td>
<td>58.2 Bb</td>
<td>83.9 Ab</td>
</tr>
<tr>
<td>GI</td>
<td>96.1 Aab</td>
<td>107 Aa</td>
<td>116 Aa</td>
<td>92.6 Aa</td>
<td>106 Aa</td>
<td>99.6 Aab</td>
</tr>
<tr>
<td>GM</td>
<td>98.6 Aa</td>
<td>96.0 Aa</td>
<td>100 Aa</td>
<td>86.2 Aa</td>
<td>92.7 Aa</td>
<td>97.5 Aab</td>
</tr>
<tr>
<td>GV</td>
<td>82.8 Bb</td>
<td>98.0 ABa</td>
<td>118 Aa</td>
<td>85.1 Ba</td>
<td>101 ABa</td>
<td>110 Aa</td>
</tr>
</tbody>
</table>

NAMF refers to the control with no AMF inoculation. GI refers to *Glomus intraradices*; GM refers to *Glomus mosseae*; GV refers to *Glomus versiforme*. Values in the same column with the same small letter are not significantly different among different AMF species. Values in the same row with the same capital letter are not significantly different in the same BDE-209 treatment.
(You et al., 1999), leading to desorption of organic matter from solid surfaces and soil particle dispersion due to the repulsion reaction (You et al., 1999). According to Table 3.5, the sand with AMF inoculation showed the significantly higher DOC concentrations than non-mycorrhizal treatments. The elevated DOC content in sand might be due to the increased root exudates in the rhizosphere which could be attributed to the enhanced plant growth and rhizospheric activity by rice root-fungi symbiosis (Cardon and Whitbeck, 2007). The root colonization rate was significantly correlated with both the root lipid content and DOC content of soil of Hefengzhan treatment ($r = 0.622$, $p < 0.01$ and $r = 0.602$, $p < 0.01$, respectively). These suggest that the increase in mycorrhizal colonization of rice root prominently promoted the root lipid content of rice and DOC content of soil.

3.3.4 Contribution of AMF to Dissipation and Degradation of BDE-209 by Rice Cultivars

In the BDE-209 treatment of various rice cultivars under the interference of AMF at different pHs, the dissipation of BDE-209 in sand varied among different rice cultivars and AMF treatments (Table 3.6). There was no BDE-209 detected in the sand and rice plants in the un-spiked controls. In the unplanted controls, there was no significant difference in concentrations of BDE-209 in sand among various pH treatments ($p > 0.05$) (Table 3.6). Comparing with the unplanted
Table 3.6 Concentration of BDE-209 in sand planted with different rice cultivars under the interference of AMF at different pHs.

<table>
<thead>
<tr>
<th>Treatments</th>
<th>Fengmeizhan (BDE-209)</th>
<th>Hefengzhan (BDE-209)</th>
<th>Guangyinzhan (BDE-209)</th>
</tr>
</thead>
<tbody>
<tr>
<td>pH 6 (Unplanted)</td>
<td>74.9 Aa</td>
<td>74.9 Aa</td>
<td>74.9 Aa</td>
</tr>
<tr>
<td>pH 6 (NAMF)</td>
<td>48.3 ABbc</td>
<td>46.2 Bb</td>
<td>50.1 Ab</td>
</tr>
<tr>
<td>pH 6 (GI)</td>
<td>46.2 Ac</td>
<td>38.5 Bb</td>
<td>41.5 ABc</td>
</tr>
<tr>
<td>pH 6 (GM)</td>
<td>51.6 Ab</td>
<td>41.8 ABb</td>
<td>39.6 Bc</td>
</tr>
<tr>
<td>pH 6 (GV)</td>
<td>48.7 Abc</td>
<td>45.4 Ab</td>
<td>47.2 Ab</td>
</tr>
<tr>
<td>pH 7 (Unplanted)</td>
<td>75.3 Aa</td>
<td>75.3 Aa</td>
<td>75.3 Aa</td>
</tr>
<tr>
<td>pH 7 (NAMF)</td>
<td>40.2 Ab</td>
<td>35.6 Bb</td>
<td>41.0 Ab</td>
</tr>
<tr>
<td>pH 7 (GI)</td>
<td>32.5 Ac</td>
<td>27.5 Bc</td>
<td>29.1 ABd</td>
</tr>
<tr>
<td>pH 7 (GM)</td>
<td>40.4 Ab</td>
<td>34.7 Bb</td>
<td>36.0 Bc</td>
</tr>
<tr>
<td>pH 7 (GV)</td>
<td>41.5 Ab</td>
<td>33.5 Bb</td>
<td>41.7 Ab</td>
</tr>
<tr>
<td>pH 8 (Unplanted)</td>
<td>75.4 Aa</td>
<td>75.4 Aa</td>
<td>75.4 Aa</td>
</tr>
<tr>
<td>pH 8 (NAMF)</td>
<td>54.8 Ab</td>
<td>47.9 Bb</td>
<td>50.0 ABb</td>
</tr>
<tr>
<td>pH 8 (GI)</td>
<td>47.3 Ab</td>
<td>42.6 ABb</td>
<td>38.2 Bd</td>
</tr>
<tr>
<td>pH 8 (GM)</td>
<td>51.1 Ab</td>
<td>45.2 Ab</td>
<td>46.2 Ac</td>
</tr>
<tr>
<td>pH 8 (GV)</td>
<td>53.2 Ab</td>
<td>49.3 Ab</td>
<td>50.1 Ab</td>
</tr>
</tbody>
</table>

Unplanted refers to unplanted controls. NAMF refers to the control with no AMF inoculation. GI refers to *Glomus intraradices*; GM refers to *Glomus mosseae*; GV refers to *Glomus versiforme*. Values with the same small letter are not significantly different among different AMF species in the same pH treatment. Values with the same capital letter are not significantly different in the same row according to one-way ANOVA test.
controls, the dissipation of BDE-209 in sand was significantly promoted with the planted rice cultivars (Table 3.6), which was in line with the results mentioned in Section 3.3.1 (p < 0.05). This evaluated the ability of the rice plants in dissipation of BDE-209 in sand. Generally, the sand planted with Hefengzhan showed the lowest concentration of BDE-209 (e.g. pH 7 (NAMF) = 35.6 nmol/kg) among the three rice cultivars (p < 0.05). On the other hand, inoculation of AMF (especially GI) significantly increased the degradation of BDE-209 in sand, when compared with the sand without AMF (e.g. Hefengzhan at pH 7 (GI) = 27.5 nmol/kg) (p < 0.05). There was a significant correlation between the concentrations of BDE-209 in sand and root colonization rates (r = -0.307, p < 0.05), but it did not significantly correlated with DOC content in sand (p > 0.05). Wang et al. (2011) also demonstrated a significant positive correlation between BDE-209 dissipation rate in soil and the signature fatty acid of AM fungi (16:1ω5t). In symbiosis, the host plant provides a growing environment for mycorrhizal fungi by supplying carbon as energy source in root exudates and the fungi will promote growth of the plant in return (Pinton et al., 2007). The root exudates include a wide range of organic compounds, such as polysaccharides, amino acids, fatty acids, growth factors and enzymes (Pinton et al., 2007). It was reported that PBDEs could be degraded by nitroreductase (NaR) and glutathione-transferase (GST) in the root
crude enzyme extracts from Italian ryegrass, pumpkin, and maize (Huang et al., 2013). This might suggest that the increased degradation of BDE-209 in sand with GI inoculation treatment might be due to the enhanced plant growth and production of root exudates with PBDEs-degrading enzymes, such as NaR and GST in rice.

The interactions of pH change and BDE-209 dissipation in sand were similar among the three rice cultivars (Table 3.6). Consequently, only the relationships between pHs, BDE-209 in sand and BDE-209 in plant tissues in the treatment of Hefengzhan are illustrated in Fig. 3.3 due to the greatest BDE-209 degradability of Hefengzhan after 60-day cultivation. The least amount of BDE-209 was observed at pH 7 in the non-AMF, GI and GV treatments (p < 0.05), while there was no significant difference in BDE-209 concentrations in sand with pH changes in the unplanted control (p > 0.05) (Fig. 3.3). This phenomenon was presented in both mycorrhizal and non-mycorrhizal treatments, indicating that the variation in BDE-209 concentrations was mainly due to the degradation response of the rice plant to BDE-209 at different pHs but not the interference of AMF on the rice plant. This indicated that pH 7 flavored the dissipation and degradation of BDE-209 in sand by the rice cultivars. Although it was evident that DOC was the highest in sand at pH 8 which might facilitate the dispersion of organic matter
Figure 3.3 Dissipation and accumulation of BDE-209 in sand and plant tissues (roots and shoots) of Hefengzhan under the interference of AMF at different pHs. (a) Concentration of BDE-209 in sand, (b) in roots and (c) in shoots of rice. Unplanted refers to unplanted controls. Non AMF refers to the control with no AMF inoculation. GI refers to *Glomus intraradices*; GM refers to *Glomus mosseae*; GV refers to *Glomus versiforme*. Bars with the same letter at the top were not significantly different (p > 0.05) in the same AMF treatment according to one-way ANOVA test.
adsorbed with BDE-209 and made BDE-209 more bioavailable to the rice plants (You et al., 1999; Sverdrup et al., 2003), the greatest dissipation rate of BDE-209 in sand (63.4%) was not observed at pH 8 but pH 7. In addition, it was found that the concentration of BDE-209 in sand was not significantly correlated with DOC content in sand ($r = -0.078$, $p > 0.05$), so there might be other factors (other than DOC) mainly mediated the dissipation of BDE-209 in sand.

3.3.5 Contribution of AMF to Uptake and Accumulation of BDE-209 in Rice

Tables 3.7 and 3.8 list the concentrations of BDE-209 accumulated in roots and shoots of rice cultivars under the interference of AMF and different pH levels after 60-day cultivation. The results were coincident with those observed in sand. Inoculation of AMF enhanced the uptake of BDE-209 in roots (e.g. Hefengzhan (BDE-209) at pH 7 (GI) = 28.3 nmol/kg) ($p < 0.05$) (Table 3.7). However, there was no observable increase of BDE-209 in shoots of rice cultivars inoculated with AMF ($p > 0.05$) (Table 3.8). In both roots and shoots, Hefengzhan showed the greatest ability in accumulation of BDE-209 among the three rice cultivars ($p < 0.05$). In the analysis of the effect of pHs on BDE-209 accumulation in plant tissues (roots and shoots) of Hefengzhan, the effect of pH 7 on accumulation of BDE-209 in plant tissues was less prominent than in sand (e.g. shoot of Hefengzhan (BDE-209) at pH 7 (GI) = 0.427 nmol/kg) (Fig. 3.3). In Section 3.3.3,
Table 3.7 Concentrations of BDE-209 in roots under the interference of AMF at different pHs.

<table>
<thead>
<tr>
<th>Treatments</th>
<th>Fengmeizhan (BDE-209)</th>
<th>Hefengzhan (BDE-209)</th>
<th>Guangyinzhan (BDE-209)</th>
</tr>
</thead>
<tbody>
<tr>
<td>pH6 (NAMF)</td>
<td>15.0 Bb</td>
<td>18.3 Ac</td>
<td>16.2 ABb</td>
</tr>
<tr>
<td>pH 6 (GI)</td>
<td>19.0 Ba</td>
<td>26.9 Aa</td>
<td>20.3 Ba</td>
</tr>
<tr>
<td>pH 6 (GM)</td>
<td>19.2 Ba</td>
<td>23.4 Ab</td>
<td>19.0 Bab</td>
</tr>
<tr>
<td>pH 6 (GV)</td>
<td>16.8 Bab</td>
<td>22.1 Ab</td>
<td>18.5 Bab</td>
</tr>
<tr>
<td>pH 7 (NAMF)</td>
<td>15.9 Bb</td>
<td>18.9 Ac</td>
<td>17.9 ABb</td>
</tr>
<tr>
<td>pH 7 (GI)</td>
<td>20.2 Ba</td>
<td>28.3 Aa</td>
<td>21.6 Ba</td>
</tr>
<tr>
<td>pH 7 (GM)</td>
<td>20.5 Ba</td>
<td>25.3 Ab</td>
<td>20.5 Ba</td>
</tr>
<tr>
<td>pH 7 (GV)</td>
<td>18.0 Cab</td>
<td>23.8 Ab</td>
<td>21.8 Ba</td>
</tr>
<tr>
<td>pH 8 (NAMF)</td>
<td>15.0 Bb</td>
<td>17.2 Ac</td>
<td>15.9 Bb</td>
</tr>
<tr>
<td>pH 8 (GI)</td>
<td>19.3 Ba</td>
<td>23.6 Aa</td>
<td>20.1 Ba</td>
</tr>
<tr>
<td>pH 8 (GM)</td>
<td>19.1 Ba</td>
<td>23.9 Aa</td>
<td>18.9 Ba</td>
</tr>
<tr>
<td>pH 8 (GV)</td>
<td>16.1 Bb</td>
<td>20.5 Ab</td>
<td>18.7 ABA</td>
</tr>
</tbody>
</table>

NAMF refers to the control with no AMF inoculation. GI refers to *Glomus intraradices*; GM refers to *Glomus mosseae*; GV refers to *Glomus versiforme*. Values with the same small letter are not significantly different among different AMF species in the same pH treatment. Values with the same capital letter are not significantly different in the same row according to one-way ANOVA test.
Table 3.8 Concentrations of BDE-209 in shoots under the interference of AMF at different pHs.

<table>
<thead>
<tr>
<th>Treatments</th>
<th>Fengmeizhan (BDE-209)</th>
<th>Hefengzhan (BDE-209)</th>
<th>Guangyinzhan (BDE-209)</th>
</tr>
</thead>
<tbody>
<tr>
<td>pH 6 (NAMF)</td>
<td>0.257 Ba</td>
<td>0.385 Aa</td>
<td>0.251 Ba</td>
</tr>
<tr>
<td>pH 6 (GI)</td>
<td>0.286 Ba</td>
<td>0.418 Aa</td>
<td>0.270 Ba</td>
</tr>
<tr>
<td>pH 6 (GM)</td>
<td>0.271 ABa</td>
<td>0.315 Ab</td>
<td>0.245 Ba</td>
</tr>
<tr>
<td>pH 6 (GV)</td>
<td>0.258 Ba</td>
<td>0.295 Ab</td>
<td>0.254 Ba</td>
</tr>
<tr>
<td>pH 7 (NAMF)</td>
<td>0.315 Ba</td>
<td>0.409 Aab</td>
<td>0.324 Ba</td>
</tr>
<tr>
<td>pH 7 (GI)</td>
<td>0.344 Ba</td>
<td>0.427 Aa</td>
<td>0.365 ABa</td>
</tr>
<tr>
<td>pH 7 (GM)</td>
<td>0.319 Ba</td>
<td>0.402 Aab</td>
<td>0.320 Ba</td>
</tr>
<tr>
<td>pH 7 (GV)</td>
<td>0.282 Ba</td>
<td>0.352 Ab</td>
<td>0.322 ABa</td>
</tr>
<tr>
<td>pH 8 (NAMF)</td>
<td>0.259 Ba</td>
<td>0.323 Aa</td>
<td>0.251 Ba</td>
</tr>
<tr>
<td>pH 8 (GI)</td>
<td>0.273 Ba</td>
<td>0.352 Aa</td>
<td>0.256 Ba</td>
</tr>
<tr>
<td>pH 8 (GM)</td>
<td>0.265 Ba</td>
<td>0.369 Aa</td>
<td>0.261 Ba</td>
</tr>
<tr>
<td>pH 8 (GV)</td>
<td>0.261 Ba</td>
<td>0.328 Aa</td>
<td>0.248 Ba</td>
</tr>
</tbody>
</table>

NAMF refers to the control with no AMF inoculation. GI refers to *Glomus intraradices*; GM refers to *Glomus mosseae*; GV refers to *Glomus versiforme*. Values with the same small letter are not significantly different among different AMF species in the same pH treatment. Values with the same capital letter are not significantly different in the same row according to one-way ANOVA test.
GI showed the greatest performance in root colonization rates, enhancement of biomass of rice cultivars, and root lipid contents. There was a positive significant correlation between the concentrations of BDE-209 in plant tissues (roots and shoots) and the biomass of plant tissues of Hefengzhan (sum of the dry weight of roots and shoots) \( (r = 0.849, p < 0.01) \). Positive significant correlations were also found between BDE-209 concentration in roots and root lipid contents of AMF inoculated rice roots, and also between the concentration of BDE-209 in plant tissues (roots and shoots) of Hefengzhan and the root colonization rates, respectively \( (r = 0.425, p < 0.05; r = 0.731, p < 0.01) \). This suggested that the concentration of BDE-209 in rice roots was closely related with the root lipid contents of AMF inoculated roots of Hefengzhan. This finding agreed with those stated in Section 3.3.2 and also results obtained by Huang et al. (2010). The present results also revealed an increase in the accumulated BDE-209 in plant tissues (roots and shoots) of Hefengzhan with the root colonization rates.

3.3.6 Contribution of AMF to Debromination of BDE-209 in Sand and Rice Plants

The PBDE congeners profiles of sand and plant tissues (roots and shoots) of Hefengzhan are illustrated in Fig. 3.4. Comparing with the non-inoculated control, inoculation of AMF slightly increased the number of PBDE congeners in sand and
Figure 3.4 Degradation profiles of BDE-209 in sand and plant tissues (roots and shoots) of Hefengzhan under the interference of AMF at different pHs. (a) PBDE congeners profile in sand, (b) in roots and (c) in shoots of Hefengzhan. NAMF refers to the control with no AMF inoculation. GI refers to *Glomus intraradices*; GM refers to *Glomus mosseae*; GV refers to *Glomus versiforme*. 
plant tissues. This was consistent with the results described in Wang et al. (2011). This indicated debromination of BDE-209 in the AMF inoculated rice treatment was enhanced when compared with the non-inoculated control treatment. BDE-209 was the dominant congener in the sand and root samples but not in the shoots samples (e.g. occupied 57.0% in sand; occupied 50.1% in roots; occupied 8.49% in shoots of Hefengzhan (GI) at pH 7, respectively) (Fig. 3.4). Wang et al. (2011) suggested that the molecules of BDE-209 were too big for further translocation into the shoots. The congener distributions in AMF inoculated shoot samples were relatively more even than those in sand and root samples (Fig. 3.4). Hexa- occupied the majority (e.g. 20.4% in shoots of Hefengzhan at pH 7 (GI)) of the profile. This might be due to the further degradation of the higher brominated PBDEs, such as nona-BDEs, octa-BDEs and hepta-BDEs.

3.3.7 Partition-limited Model Analysis of Rice Uptake of BDE-209

A partition-limited model was applied for estimating and describing the approach of the uptake of BDE-209 by rice in sand (Chiou et al., 2001). Equation 1 was employed for the calculation (Section 3.2.8). Octanol was assimilated to biological lipids in contaminant partition (Chiou, 2001). Therefore, the partition coefficient with dried root organic matter ($K_{pom}$) of BDE-209 in rice, which is one of the components of the function, was assumed to be the same as the $K_{ow}$ value.
The specific $K_{som}$ (or $K_{oc}$) (Chiou, 2002) of BDE-209 was derived from the equation 5 (Zou et al., 2007) as follows:

$$\log K_{oc} = 0.75 \times \log K_{ow} - 0.35$$

(3.5)

In the interaction of organic contaminants and plant seedlings, quasi-equilibrium partition model can be applied to estimate the extent of a particular contaminant to reach equilibrium between plant and external water at a specific point of time (Chiou et al., 2001). The movement of the contaminant molecules into the plant can be described by the magnitude of quasi-equilibrium factors ($\alpha_{pt}$) of roots (Equation 1). The quasi-equilibrium factors could be quantified from the $K_{pom} \approx K_{ow}$ (Chiou, 2001) and measured $f_{pw}$ (0.942) and $f_{pom}$ values (0.0579). It was found that the $\alpha_{pt}$ value of BDE-209 based on the concentrations in sand and rice roots (Hefengzhan = $1.15 \times 10^{-4} \pm 2.78 \times 10^{-6}$) was also smaller than 1, implying a non-equilibrium state of movement of the molecules and a passive transport dominated uptake approach (Chiou et al., 2001). The result was in line with the findings in ryegrass involving uptake of phenanthrene and pyrene (Gao et al., 2005). These elucidated that the transport of BDE-209 into rice roots from sand is also a non-equilibrium and passive movement. In addition, the movement of BDE-209 into rice roots in sand (lower $\alpha_{pt}$ value) was likely further away from equilibrium than PCB and DDT (Table
3.9), which have similar chemical structure as PBDEs but with lower log $K_{ow}$ values (Rahman et al., 2001). One of the reasons for this might be due to the higher log $K_{ow}$ value of BDE-209 (9.97).

3.3.8 Uptake Pathway of BDE-209 in Rice Roots

Figure 3.5 shows that the concentrations of BDE-209 in fresh and dead rice roots increased proportionally with the concentrations of BDE-209 in the external solutions. All the concentration-dependent uptake curves of BDE-209 in different rice cultivars were in linear shape (Fig. 3.5). The uptake coefficients (the ratio of BDE-209 concentrations in roots to those in external solution) of BDE-209 in different rice cultivars were generally higher in dead roots than those in fresh roots. It revealed a dominant apoplastic pathway (through cell walls and intercellular spaces) in the uptake of BDE-209 by rice roots (Wild et al., 2005). Active transport of organic compounds uptake was prohibited after killing the roots by heat, so lipid–like substance in roots would be essential in partitioning of BDE-209 into the root cells (Su and Zhu, 2007). On the other hand, the relatively greater uptake coefficients of fresh Hefengzhan roots among the three rice cultivars might be due to the metabolic loss of BDE-209 in the external solution.

According to the results of visualization of PAHs uptake by maize and wheat roots, apoplastic flow via cell walls dominated the compounds movement within
Table 3.9 The quasi-equilibrium factors ($\alpha_{q\theta}$) of different compounds in various plant species and media.

<table>
<thead>
<tr>
<th>Compounds</th>
<th>$\log K_{ow}$</th>
<th>Plant species</th>
<th>Medium</th>
<th>$\alpha_{q\theta}$</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Arazine</td>
<td>2.71</td>
<td>Barley plants (<em>Hordeum vulgare cv. Georgie</em>)</td>
<td>soil (3.5% in SOM)</td>
<td>1</td>
<td>Chiou et al., 2001</td>
</tr>
<tr>
<td>Phenanthrene</td>
<td>4.46</td>
<td>Ryegrass (<em>Lolium multiflorum Lam</em>)</td>
<td>soil (1.5% in SOM)</td>
<td>$8.1 \times 10^{-3}$</td>
<td>Gao et al., 2005</td>
</tr>
<tr>
<td>Pyrene</td>
<td>4.88</td>
<td>Ryegrass (<em>Lolium multiflorum Lam</em>)</td>
<td>soil (1.5% in SOM)</td>
<td>$12 \times 10^{-3}$</td>
<td>Gao et al., 2005</td>
</tr>
<tr>
<td>Hexachlorobenzene</td>
<td>5.50</td>
<td>Barley plants (<em>Hordeum vulgare cv. Georgie</em>)</td>
<td>soil (3.5% in SOM)</td>
<td>0.2</td>
<td>Chiou et al., 2001</td>
</tr>
<tr>
<td>2,4,6,2',4'-Pentachlorobiphenyl (PCB)</td>
<td>5.92</td>
<td>Barley plants (<em>Hordeum vulgare cv. Georgie</em>)</td>
<td>soil (3.5% in SOM)</td>
<td>0.19</td>
<td>Chiou et al., 2001</td>
</tr>
<tr>
<td>Dichlorodiphenyltrichloroethane (DDT)</td>
<td>6.36</td>
<td>Barley plants (<em>Hordeum vulgare cv. Georgie</em>)</td>
<td>soil (3.5% in SOM)</td>
<td>0.11</td>
<td>Chiou et al., 2001</td>
</tr>
<tr>
<td>Decabromodiphenyl ether (BDE-209)</td>
<td>9.97</td>
<td>Rice-Fengmeizhan (<em>Oryza sativa</em>)</td>
<td>soil (0.88% in SOM)</td>
<td>$0.099 \times 10^{3}$</td>
<td>ATSDR, 2004b; This study</td>
</tr>
<tr>
<td>Decabromodiphenyl ether (BDE-209)</td>
<td>9.97</td>
<td>Rice-Hefengzhan (<em>Oryza sativa</em>)</td>
<td>soil (0.88% in SOM)</td>
<td>$0.115 \times 10^{3}$</td>
<td>ATSDR, 2004b; This study</td>
</tr>
<tr>
<td>Decabromodiphenyl ether (BDE-209)</td>
<td>9.97</td>
<td>Rice-Guangyinzhan (<em>Oryza sativa</em>)</td>
<td>soil (0.88% in SOM)</td>
<td>$0.123 \times 10^{3}$</td>
<td>ATSDR, 2004b; This study</td>
</tr>
</tbody>
</table>
Figure 3.5 Uptake isotherms of BDE-209 with fresh and dead rice roots of (a) Fengmeizhan (b) Hefengzhan and (c) Guangyinzhan. Error bars denote the standard errors of the means (n=3).
the cortex, with only a small portion transported into cellular vacuoles through symplastic flow (Wild et al., 2005). The results of the current study were consistent with this study. The greater capacity of dead roots for sorption of BDE-209 could be explained by the enhanced permeability of the cell membrane after heating the fresh roots (Su and Zhu, 2007). Consequently, more BDE-209 was partitioned into the organic substrate of the root cells, promoting the uptake of the compound.

3.4 Conclusion

The present study investigated the uptake and translocation of BDE-209 by three rice cultivars namely Fengmeizhan, Hefengzhan and Guangyinzhan associated with AMF (GI, GM and GV) at different pHs. The uptake mechanisms and pathway of BDE-209 by rice roots were also elucidated by application of the partition-limited model and sorption analysis. The rice inoculated by AMF was promoted in uptake and debromination of BDE-209. More lower brominated congeners were detected in shoots than roots implying further metabolism in the soil-plant system. All three rice cultivars could enhance the degradation of BDE-209 in sand, especially Hefengzhan showed the greatest dissipation and accumulation of BDE-209 in sand and plant tissues at pH 7, which agreed with the hypothesis. According to the quasi-equilibrium factors (αpt), the uptake of
BDE-209 by rice roots in sand was in a state of passive transport dominated uptake approach. In addition, the apoplastic pathway dominated the transport of BDE-209 in rice roots, indicating the importance of the cell wall in the plant uptake of these organic contaminants.
CHAPTER 4
UPTAKE, TRANSLOCATION AND TRANSPORT MECHANISMS OF BDE-47 AND BDE-99 IN RICE (ORYZA SATIVA) ASSOCIATED WITH ARBUSCULAR MYCORRHIZAL FUNGI

4.1 Introduction

The commercial PBDEs were usually manufactured as three technical mixtures: penta-, octa-, and deca-products, in which the penta-product accounted 6% of the global consumption in 2001 (Meng et al., 2007). The commercial penta-mixture has been used in non-foamed PUR in casings, electronic equipment and PUR foam for cushioning in upholstery (ATSDR, 2004b; UNEP, 2007). The penta-product mixture contains 2,2’,4,4’-tetrabromodiphenyl ether (BDE-47), 2,2’,4,4’,5-pentabromodiphenyl ether (BDE-99), 2,2’,4,4’,6-pentabromodiphenyl ether (BDE-100), 2,2’,4,4’,5,5’- hexabromodiphenyl ether (BDE-153), and 2,2’,4,4’,5,6’-tetrabromo-diphenyl ether (BDE-154) in which BDE-47 (28%) and -99 (43%) as two major components, which are also two major congeners detected in biota and environmental samples (US EPA, 2008a; Hites, 2004). Due to the potential deterioration to the environment and organisms, the use of
commercial penta-BDE was banned in all applications for the EU market in 2004, followed by China, where penta-BDE has been banned for application in electric and electronic products (BSEF, 2013). Although the applications of penta-BDEs seem to be phased out after the adoption of the banning or ceasing legislation, the release of BDE-99 into the ambient environment can still be possible through degradation of deca-BDE (Rahman et al., 2001).

BDE-47 and -99 belong to tetra- and penta-bromodiphenyl ether groups which contain four and five bromine atoms on the two phenyl rings, respectively (Fig. 4.1) (US EPA, 2008a, b). Uncontrolled recycling and dismantling activities of e-wastes have rendered soil a major receptor of hazardous contaminants (e.g. BDE-47 and -99) contained in these appliances (Leung et al., 2007). High concentrations (129 and 333 ng/g, dry wt, respectively) of BDE-47 and -99 were reported in the soil of a printer roller dumping site in Guiyu, China (e-waste recycling site), which was probably attributed to the use of commercial penta-BDE in the manufacture of electronic appliances (Leung et al., 2007).

BDE-47 and -99 are two dominant congeners frequently detected in human body especially adipose tissue, liver, milk, and blood due to their high $K_{ow}$ values (6.81 and 7.32) (Wang et al., 2007; US EPA, 2008a, b). For example, BDE-47 and -99 were detected in hair (40.1 and 17.3 ng/g dw), and breast milk samples (27.5
Figure 4.1 Chemical structures of BDE-47 and -99 (Fang et al., 2008).
and 10.8 ng/g fat) from Taizhou, China (Leung et al., 2010). Due to the persistence property of BDE-47 and -99, the commercial penta-BDE might stay in human body relatively longer than deca-BDE (BDE-209) did, resulting in higher health risk after exposure (ATSDR, 2004a). Despite of the uncertainty of BDE-47 and -99 in carcinogenicity in human (US EPA, 2008a, b), their exposure was believed to be related with oxidative injury in human cells and neurotoxic effects (Souza et al., 2013; Tagliaferri et al., 2010).

In addition to the frequent occurrence of BDE-99 and -47 in biota and environmental samples, they were also commonly found in the products of TiO₂ photocatalysis of BDE-209 by HA/visible light (Chapter 2). Based on these, there is a need for studying the uptake behavior of these two PBDE congeners in rice cultivar associated with AMF, so as to provide more information about the bioavailability of lower molecular PBDE congeners for the subsequent combination of photocatalysis and phytoremediation technology for treating PBDEs at the end of the present study. Consequently, the uptake and fate of these two congeners in Hefengzhan, which was the rice cultivar manifested with the highest ability in degradation and accumulation of BDE-209 (Chapter 3), will be investigated in this Chapter.

In order to mitigate the environmental and health effects of PBDEs,
phytoremediation has been used for restoration of PBDEs-contaminated sites. However, there are limited studies concerning the uptake of BDE-47 and -99 by plants. The study conducted by Mueller et al. (2006) was one of the limited studies about the phytoremediation of BDE-47 and -99 in contaminated soil. The uptake of a commercial penta-BDE mixture (DE-71) (75μg/kg), including BDE-47, BDE-99 and BDE-100, by small round red cherriette (*Raphanus sativus*) and yellow gold rush (*Cucurbita sp.*) was investigated (Mueller et al., 2006). It was found that the BDE-47 left in planted and unplanted soils after 10 weeks was apparently higher than those of BDE-99 and BDE-100 (Mueller et al., 2006). This implied that BDE-47 was more recalcitrant to degradation or plant uptake than BDE-99 and BDE-100. Huang et al. (2011) also noted higher portions of BDE-47 in roots of pumpkin, maize and ryegrass than those in soil. This revealed that BDE-47 was readily taken up and accumulated by roots of these plants. The translocation factor (*C*/<sub>stem</sub>/*C*/<sub>root</sub>) (<1) of BDE-47 was much lower than all those of BDE-15 and BDE-28 (Wang et al., 2011), which indicating that BDE-47 tended to distribute in root rather than stem. They also noted the debromination of BDE-47 might result in the production of BDE-3, BDE-15 and BDE-28. BDE-99 was able to be taken up and accumulated by plants (Italian ryegrass, pumpkin, maize, tobacco, nightshade and maize) (Huang et al., 2011; Vrkoslavová et al.,
Vrkoslavová et al. (2010) noted that the treatments with plants (tobacco and nightshade) enhanced the dissipation of BDE-99 in contaminated sewage sludge obtained from wastewater treatment plant in Hradec Králové, Czech Republic. The reduction percentages of BDE-99 in tobacco and nightshade treatments were 6.29% and 6.83%, respectively (Vrkoslavová et al., 2010). Nonetheless, these studies did not provide sufficient information about the uptake mechanisms and pathways of BDE-47 and -99 in plants. Their uptakes by rice plant (Hefengzhan) could be estimated and described by a partition-limited model proposed by Chiou et al. (2001).

Our results from previous experiments (Chapter 3) demonstrated the ability of AMF (particular *Glomus intraradices*) in enhancing the degradation and accumulation of BDE-209 by rice plants. It was also reported that *Glomus mosseae* exerted positive effects on the uptake and accumulation of lower brominated PBDEs (di- through nona-BDEs) in ryegrass (Wang et al., 2011). It seems apparent that AMF are beneficial to their host plants by enhancing the release of root exudation, and might help for the degradation of BDE-209 (Pinton et al., 2007; Huang et al., 2013).

Based on the above information, the present study aimed at examining the fate, degradation and uptake mechanisms of BDE-47 and -99 in rice cultivar
It was hypothesized that degradation of BDE-47 and -99 might be enhanced by planting with Hefengzhan but in lower reduction rate than those of BDE-209 associated with AMF. The major objectives of the present experiment were to investigate (1) the fate of BDE-47 and -99 in the soil-plant system of rice (Hefengzhan); (2) the influences of different soil pH levels on the degradation and uptake of BDE-47 and -99 by rice; (3) the interference of mycorrhizal roots through inoculation of AMF on the degradation of BDE-47 and -99 in sand; and (4) the uptake mechanisms and pathways of BDE-47 and -99 in rice roots.

4.2 Materials and Methods

The experimental setup of the present study was the same as Chapter 3, except BDE-47 (2,2’,4,4’-tetrabromodiphenyl ether, > 98%) (Chem Service, Inc., USA) and BDE-99 (2,2’,4,4’,5-pentabromodiphenyl ether) (AccuStandard Inc., USA) were used instead of BDE-209, and Hefengzhan which showed the best performance in accumulation and degradation of BDE-209 was employed. The extraction of PBDEs, chemical analyses methods and data analyses were the same as those described in Chapter 3.

4.3 Results and Discussion

4.3.1 Contribution of AMF to Growth of Rice in BDE-47 and -99 Contaminated Sand
Hefengzhan was found to possess the greatest ability in degradation and accumulation of BDE-209, among the three rice cultivars (Chapter 3, Sections 3.3.1 and 3.3.2). Therefore, Hefengzhan was chosen for further investigation of the uptake of BDE-47 and -99 by rice plants inoculated with AMF. Table 4.1 illustrates the mycorrhizal infection rates of Hefengzhan after 60-day cultivation. The root colonization rates of the non-inoculated rice roots (control) showed no colonization, while those of inoculated rice roots varied among AMF species and pH levels (Table 4.1). There was no significant difference in root colonization rates between all PBDEs spiked and non-spiked treatments (p > 0.05), indicating the addition of BDE-47 and -99 into the sand did not affect colonization rates of AMF (Table 4.1). This result was in line with that presented in Chapters 3, implying that BDE-47 and -99 in concentrations of 73.8 and 74.6 µg/kg, respectively, did not exert significant effects on the AMF colonization rates of rice roots.

On the other hand, changes in pH levels only led to observable effects on the root colonization rate of GI inoculated rice roots, but not other treatments (Table 4.1). This might suggest that GI was more susceptible to the high pH (pH 8) (BDE-47 ctrl (GI) = 14.6% ; BDE-99 ctrl (GI) = 14.4%) than the other two AMF (GM and GV), leading to a decrease in root colonization rates, reflected by the
Table 4.1 Mycorrhizal infection rates of rice (Hefengzhan) roots (% of total root length infected) at different pH levels.

<table>
<thead>
<tr>
<th>Treatments</th>
<th>pH 6 (ctrl)</th>
<th>pH 7 (ctrl)</th>
<th>pH 8 (ctrl)</th>
<th>pH 6 (PBDEs)</th>
<th>pH 7 (PBDEs)</th>
<th>pH 8 (PBDEs)</th>
</tr>
</thead>
<tbody>
<tr>
<td>BDE-47 (GI)</td>
<td>16.1 ABa</td>
<td>17.2 Aa</td>
<td>14.6 Ba</td>
<td>15.9 Aa</td>
<td>16.3 Aa</td>
<td>14.4 Aa</td>
</tr>
<tr>
<td>BDE-47 (GM)</td>
<td>11.6 Ab</td>
<td>11.9 Ab</td>
<td>10.4 Ab</td>
<td>12.6 Aa</td>
<td>12.9 Aa</td>
<td>12.4 Aa</td>
</tr>
<tr>
<td>BDE-47 (GV)</td>
<td>3.09 Ac</td>
<td>3.21 Ac</td>
<td>3.03 Ac</td>
<td>2.99 Ab</td>
<td>3.28 Ab</td>
<td>2.83 Ab</td>
</tr>
<tr>
<td>BDE-99 (GI)</td>
<td>15.8 Aa</td>
<td>16.3 Aa</td>
<td>14.4 Ba</td>
<td>15.5 Aa</td>
<td>15.9 Aa</td>
<td>13.2 Ba</td>
</tr>
<tr>
<td>BDE-99 (GM)</td>
<td>12.8 Aa</td>
<td>11.3 Ab</td>
<td>10.4 Ab</td>
<td>11.1 Ab</td>
<td>10.1 Ab</td>
<td>10.6 Ab</td>
</tr>
<tr>
<td>BDE-99 (GV)</td>
<td>2.95 Ab</td>
<td>3.03 Ac</td>
<td>2.91 Ac</td>
<td>3.27 Ac</td>
<td>3.21 Ac</td>
<td>2.91 Ac</td>
</tr>
</tbody>
</table>

Ctrl refers to non-spiking control; GI refers to *Glomus intraradices*; GM refers to *Glomus mosseae*; GV refers to *Glomus versiforme*. Values with the same small letter are not significantly different among different AMF species in the same PBDEs (BDE-47 or -99) treatment. Values with the same capital letter are not significantly different among different pH levels in the same PBDEs treatment under one-way ANOVA test (p > 0.05).
reduced arbuscule and vesicle formation in the host plant (Van Aarle et al., 2002).

In both non-spiking control and PBDEs spiked treatments, the highest colonization rates were observed in roots inoculated with GI (e.g. BDE-47 at pH 7 (GI) = 17.2%; BDE-99 at pH 7 (GI) = 16.3%) (p < 0.05). The infection rates of rice roots by the three studied AMF species under the influence of BDE-47 and -99, in descending order were GI > GM > GV (Table 4.1). This finding coincided with those from Chapter 3. Secilia and Bagyaraj (1992), also revealed that GI had the greatest performance in root colonization among the three AMF species. This might be because AMF species often varied in pH, temperature, concentration of P, water potential, presence of pollutants (e.g. heavy metals or organics) and microorganisms for optimal germination and growth (Smith and Read, 2008).

However, the root colonization rates of rice roots inoculated with the three AMF (GI, GM and GV) of the present study (both non-spiked controls and PBDEs-spiked treatments) were apparently lower than those from Secilia and Bagyaraj (1992) (GI = 39.4%; GM = 28.8%; GV = 24.8%) (Table 4.1). This could be explained by the different levels of soil organic matter between Secilia and Bagyaraj (1992) (1.23%) and the present study (0.88%), and the higher organic matter content added into soil promoted better mycorrhizal colonization (Gryndler et al., 2009). The higher content of soil organic matter (total carbon = 2.88%)
could also enhance the growth of AM hyphae (up to around 60% of increment in length) (Joner and Jakobsen, 1995). Therefore, the lower soil organic matter content of the growth substrate used in the present study might be one of the crucial factors of the lower root colonization rates of rice roots. The particularly lower colonization values from inoculation with GV also indicated the lower infectious ability of GV in the roots of Hefengzhan (Table 4.1). On the other hand, there were no significant differences between the root colonization rates in BDE-47, -99 and -209 treatments (p > 0.05), implying that the AMF had similar infectious ability to rice in BDE-47, -99 and -209 contaminated sand.

Table 4.2 illustrates the biomass of roots and shoots of Hefengzhan grown in BDE-47 and -99 contaminated sand under the influence of AMF. The results were similar to those obtained from Chapters 3. The changes of pH did not exert any significant effects on the biomass of roots (p > 0.05), but led to the lower biomass of shoot at pH 8 in the treatments with GM (BDE-47 ctrl = 7.70 g; BDE-99 (ctrl) = 7.66 g) and GV (BDE-47 (ctrl) = 6.67 g; BDE-99 (ctrl) = 5.56 g) (p < 0.05) (Table 4.2). In the treatment with spiked BDE-47, the biomass of roots and shoots was similar to their controls respectively, and there was also no significant alteration in the biomass after the addition of BDE-47 into the sand (p > 0.05). This implicated that the spiking of BDE-47 did not exert any significant effects on
plant growth, in terms of biomass.

In both control and PBDEs spiked treatments, inoculation with AMF significantly promoted the biomass of rice plants (roots and shoots) (p < 0.05), while GI generally had the best performance in roots (e.g. BDE-47 ctrl (pH 8) = 6.75 g; BDE-99 ctrl (pH 8) = 7.47 g) and shoots biomass (e.g. BDE-47 ctrl (pH 8) = 11.1 g; BDE-99 ctrl (pH 8) = 10.3 g) (Table 4.2). It was evident that GI had the greatest infectious ability and the best performance in promoting plant growth, in line with the results reported in Chapter 3. In addition, the biomass of rice cultivar (Hefengzhan) in BDE-47 and -99 treatments was significantly correlated with the root colonization rates, respectively (BDE-47: \( r = 0.717, \ p < 0.01 \); BDE-99: \( r = 0.783, \ p < 0.01 \)). This elucidated the close relationship between the root colonization and the biomass of rice plants.

The benefits of AMF on biomass enhancement of rice plant grown in BDE-209 contaminated sand had already been discussed in Chapter 3 and other studies (Secilia and Bagyaraj, 1992; Paszkowski et al., 2002). Secilia and Bagyaraj (1992) reported the abilities of AMF (include \textit{Glomus intraradices}, \textit{Glomus mosseae} and \textit{Glomus versiforme}) in enhancing the biomass of rice (Prakash) in the soil with low level of phosphate (25 kg/ha), which might be due to the increased bioavailability of some nutrients, in particular inorganic
Table 4.2 Biomass of roots and shoots of Hefengzhan under the influence of AMF.

<table>
<thead>
<tr>
<th>Treatments</th>
<th>pH 6 (ctrl)</th>
<th>pH 7 (ctrl)</th>
<th>pH 8 (ctrl)</th>
<th>pH 6 (PBDEs)</th>
<th>pH 7 (PBDEs)</th>
<th>pH 8 (PBDEs)</th>
<th>pH 6 (ctrl)</th>
<th>pH 7 (ctrl)</th>
<th>pH 8 (ctrl)</th>
<th>pH 6 (PBDEs)</th>
<th>pH 7 (PBDEs)</th>
<th>pH 8 (PBDEs)</th>
</tr>
</thead>
<tbody>
<tr>
<td>BDE-47 (NAMF)</td>
<td>4.46 Ab</td>
<td>4.98 Ab</td>
<td>4.18 Ac</td>
<td>4.21 Ab</td>
<td>4.88 Ab</td>
<td>4.27 Ab</td>
<td>6.94 Ab</td>
<td>7.57 Ac</td>
<td>7.01 Ab</td>
<td>6.35 Ab</td>
<td>6.23 Ab</td>
<td>6.77 Ab</td>
</tr>
<tr>
<td>BDE-47 (GI)</td>
<td>6.67 Aa</td>
<td>6.78 Aa</td>
<td>6.75 Aa</td>
<td>7.09 Aa</td>
<td>6.84 Aa</td>
<td>7.01 Aa</td>
<td>10.0 Aa</td>
<td>10.6 Aa</td>
<td>11.1 Aa</td>
<td>10.3 Aa</td>
<td>9.33 Aa</td>
<td>10.9 Aa</td>
</tr>
<tr>
<td>BDE-47 (GM)</td>
<td>5.62 Aab</td>
<td>5.65 Aab</td>
<td>5.35 Ab</td>
<td>5.42 Ab</td>
<td>5.54 Aab</td>
<td>5.59 Aab</td>
<td>9.33 Aa</td>
<td>9.74 Aab</td>
<td>7.70 Bb</td>
<td>8.59 ABab</td>
<td>9.26 Aa</td>
<td>7.42 Bb</td>
</tr>
<tr>
<td>BDE-47 (GV)</td>
<td>5.17 Aab</td>
<td>5.14 Ab</td>
<td>5.25 Abc</td>
<td>5.23 Ab</td>
<td>5.48 Aab</td>
<td>5.15 Aab</td>
<td>8.83 Aab</td>
<td>8.25 ABbc</td>
<td>6.67 Bb</td>
<td>8.66 Aab</td>
<td>8.81 Aa</td>
<td>6.76 Bb</td>
</tr>
<tr>
<td>BDE-99 (GI)</td>
<td>7.74 Aa</td>
<td>7.59 Aa</td>
<td>7.47 Aa</td>
<td>7.74 Aa</td>
<td>7.65 Aa</td>
<td>7.14 Aa</td>
<td>10.4 Aa</td>
<td>10.3 Aa</td>
<td>10.3 Aa</td>
<td>11.1 Aa</td>
<td>10.9 Aa</td>
<td>11.4 Aa</td>
</tr>
<tr>
<td>BDE-99 (GM)</td>
<td>6.61 Aa</td>
<td>6.64 Aa</td>
<td>6.82 Aab</td>
<td>6.55 Aab</td>
<td>6.57 Aa</td>
<td>6.62 Aa</td>
<td>8.89 AABab</td>
<td>9.45 Aab</td>
<td>7.66 Bb</td>
<td>9.30 Aab</td>
<td>9.33 Aab</td>
<td>7.67 Ab</td>
</tr>
<tr>
<td>BDE-99 (GV)</td>
<td>6.00 Aab</td>
<td>6.26 Aa</td>
<td>6.06 Aab</td>
<td>6.52 Aab</td>
<td>6.14 Aab</td>
<td>6.20 Aa</td>
<td>8.31 Ab</td>
<td>7.92 ABbc</td>
<td>5.56 Bb</td>
<td>7.68 Ab</td>
<td>7.81 Abc</td>
<td>6.31 Ab</td>
</tr>
</tbody>
</table>

Ctrl refers to non-spiking control of PBDEs treatment; NAMF refers to the non-mycorrhizal control; GI refers to *Glomus intraradices*; GM refers to *Glomus mosseae*; GV refers to *Glomus versiforme*. Values with the same small letter are not significantly different among different AMF species in the same PBDEs (BDE-47 or -99) treatment. Values with the same capital letter are not significantly different among different pHs in the same PBDEs treatment under one-way ANOVA test (p > 0.05).
(ortho)phosphate (Smith and Read, 2008). The uptake of phosphate was reported to be attributed to the expression of rice phosphate transporter gene, OsPT11, which was specifically triggered during the rice root-mycorrhizal symbiosis (Paszkowski et al., 2002). The extraradical mycelia of AMF served as functional extensions of the plant root system and enhanced the absorption of nutrients, especially phosphate (Smith et al., 2001). These elucidated the importance of AMF in acquiring phosphate for the host plant during the mycorrhizal symbiosis.

In order to further investigate the interaction between rice plant (Hefengzhan) and AMF in the BDE-47 and -99 contaminated sand, the root lipid and DOC contents in soil were analysed and the results are presented in Tables 4.3 and 4.4. Root lipid contents followed the pattern as the plant biomass (Table 4.2). There was generally no significant effect exerted on the root lipid contents by different pH levels, except those from non-inoculated controls (Table 4.3). On the other hand, spiking of BDE-47 and -99 did not result in observable differences in root lipid contents (p > 0.05) (Table 4.3). Treatments with AMF significantly increased root lipid contents in Hefengzhan (p < 0.05), particularly in the treatment with GI (BDE-47 at pH 7 = 13.1 mg/g dw; BDE-99 at pH 7 = 12.2 mg/g dw), which was significantly higher than those from the other two AMF (p < 0.05) (Table 4.3).

The root colonization rate was prominently associated with the root lipid
Table 4.3 Root lipid content of Hefengzhan under the influence of AMF.

<table>
<thead>
<tr>
<th>Treatments</th>
<th>pH 6 (ctrl)</th>
<th>pH 7 (ctrl)</th>
<th>pH 8 (ctrl)</th>
<th>pH 6 (PBDEs)</th>
<th>pH 7 (PBDEs)</th>
<th>pH 8 (PBDEs)</th>
</tr>
</thead>
<tbody>
<tr>
<td>BDE-47 (NAMF)</td>
<td>8.84 Ab</td>
<td>8.74 Ab</td>
<td>6.20 Bc</td>
<td>9.64 ABb</td>
<td>10.4 Ab</td>
<td>7.56 Bc</td>
</tr>
<tr>
<td>BDE-47 (GI)</td>
<td>12.1 Aa</td>
<td>12.6 Aa</td>
<td>11.6 Aa</td>
<td>13.0 ABa</td>
<td>13.1 Aa</td>
<td>11.9 Ba</td>
</tr>
<tr>
<td>BDE-47 (GM)</td>
<td>9.92 Aab</td>
<td>10.1 Aab</td>
<td>10.5 Aab</td>
<td>12.1 Aa</td>
<td>12.5 Aa</td>
<td>10.9 Aab</td>
</tr>
<tr>
<td>BDE-47 (GV)</td>
<td>8.76 Ab</td>
<td>9.37 Ab</td>
<td>8.50 Abc</td>
<td>9.48 Ab</td>
<td>9.38 Ab</td>
<td>8.87 Abc</td>
</tr>
<tr>
<td>BDE-99 (NAMF)</td>
<td>8.35 Ab</td>
<td>8.49 Ab</td>
<td>5.67 Bb</td>
<td>7.93 Aa</td>
<td>8.44 Ab</td>
<td>6.57 Ab</td>
</tr>
<tr>
<td>BDE-99 (GI)</td>
<td>11.4 Aa</td>
<td>11.3 Aa</td>
<td>10.9 Aa</td>
<td>10.5 Aa</td>
<td>12.2 Aa</td>
<td>11.4 Aa</td>
</tr>
<tr>
<td>BDE-99 (GM)</td>
<td>8.48 Ab</td>
<td>9.76 Aab</td>
<td>9.24 Aa</td>
<td>8.22 Aa</td>
<td>8.83 Ab</td>
<td>9.34 Aab</td>
</tr>
<tr>
<td>BDE-99 (GV)</td>
<td>7.73 Ab</td>
<td>8.70 Ab</td>
<td>8.74 Aa</td>
<td>7.49 Aa</td>
<td>8.24 Ab</td>
<td>7.44 Aab</td>
</tr>
</tbody>
</table>

Ctrl refers to non-spiking control of PBDEs treatment; NAMF refers to the non-mycorrhizal control; GI refers to *Glomus intraradices*; GM refers to *Glomus mosseae*; GV refers to *Glomus versiforme*. Values with the same small letter are not significantly different among different AMF species in the same treatment. Values with the same capital letter are not significantly different among different pHs in the same BDE-47 or -99 treatment under one-way ANOVA test (p > 0.05).
Table 4.4 Dissolved organic carbon (DOC) contents of soil planted with Hefengzhan under the influence of AMF.

<table>
<thead>
<tr>
<th>Treatments</th>
<th>DOC concentrations in soil (mg/kg)</th>
<th>pH 6 (ctrl)</th>
<th>pH 7 (ctrl)</th>
<th>pH 8 (ctrl)</th>
<th>pH 6 (PBDEs)</th>
<th>pH 7 (PBDEs)</th>
<th>pH 8 (PBDEs)</th>
</tr>
</thead>
<tbody>
<tr>
<td>BDE-47 (Unplanted)</td>
<td></td>
<td>28.3 Bb</td>
<td>35.5 ABb</td>
<td>37.5 Ac</td>
<td>29.4 Bb</td>
<td>34.4 ABb</td>
<td>40.0 Ac</td>
</tr>
<tr>
<td>BDE-47 (Non AMF)</td>
<td></td>
<td>49.2 Bb</td>
<td>52.2 Bb</td>
<td>78.7 Ab</td>
<td>43.4 Bb</td>
<td>46.1 Bb</td>
<td>80.4 Ab</td>
</tr>
<tr>
<td>BDE-47 (GI)</td>
<td></td>
<td>108 Aa</td>
<td>110 Aa</td>
<td>120 Aa</td>
<td>110 Aa</td>
<td>107 Aa</td>
<td>116 Aa</td>
</tr>
<tr>
<td>BDE-47 (GM)</td>
<td></td>
<td>98.5 Aa</td>
<td>96.6 Aa</td>
<td>98.8 Aab</td>
<td>98.0 Aa</td>
<td>98.3 Aa</td>
<td>124 Aa</td>
</tr>
<tr>
<td>BDE-47 (GV)</td>
<td></td>
<td>88.8 Ba</td>
<td>95.5 Ba</td>
<td>127 Aa</td>
<td>95.7 Aa</td>
<td>96.8 Aa</td>
<td>121 Aa</td>
</tr>
<tr>
<td>BDE-99 (Unplanted)</td>
<td></td>
<td>28.3 Bc</td>
<td>35.5 ABb</td>
<td>37.5 Ac</td>
<td>28.6 Bc</td>
<td>34.0 ABc</td>
<td>39.5 Ac</td>
</tr>
<tr>
<td>BDE-99 (Non AMF)</td>
<td></td>
<td>54.3 Bb</td>
<td>51.5 Bb</td>
<td>72.4 Ab</td>
<td>59.2 Bb</td>
<td>61.5 ABb</td>
<td>76.4 Ab</td>
</tr>
<tr>
<td>BDE-99 (GI)</td>
<td></td>
<td>106 Ba</td>
<td>112 ABa</td>
<td>119 Aa</td>
<td>117 Aa</td>
<td>103 Aa</td>
<td>109 Aa</td>
</tr>
<tr>
<td>BDE-99 (GM)</td>
<td></td>
<td>98.6 Aa</td>
<td>107 Aa</td>
<td>111 Aa</td>
<td>109 Aa</td>
<td>104 Aa</td>
<td>121 Aa</td>
</tr>
<tr>
<td>BDE-99 (GV)</td>
<td></td>
<td>95.7 Ba</td>
<td>106 A Ba</td>
<td>128 Aa</td>
<td>98.6 Ba</td>
<td>99.6 Ba</td>
<td>124 Aa</td>
</tr>
</tbody>
</table>

Ctrl refers to non-spiking control of PBDEs treatment; Unplanted refers to the unplanted control; NAMF refers to the non-mycorrhizal control; GI refers to *Glomus intraradices*; GM refers to *Glomus mosseae*; GV refers to *Glomus versiforme*. Values with the same small letter are not significantly different among different AMF species in the same treatment. Values with the same capital letter are not significantly different among different pHs in the same BDE-47 or -99 treatment. Values with asterisk (*) are significantly different from the control under independent t-test (p < 0.05).
contents, which was manifested by the significant positive correlation between these two parameters (BDE-47: \( r = 0.756, p < 0.01 \); BDE-99: \( r = 0.638, p < 0.01 \)). Cooper and Lösel (1978) studied the distribution, quantity and composition of lipid in *Glomus mosseae* infected roots of onion, clover and ryegrass and a significantly higher total lipid content was shown in the mycorrhizal infected roots (e.g. ryegrass roots = 6.6 mg/g fresh weight) than the uninfected roots (e.g. ryegrass roots = 3.7 mg/g fresh weight). The concentrations of neutral lipids (include sterol esters, triglyceride, free fatty acids, diglyceride and sterols) were generally higher in mycorrhizal infected roots than the uninfected roots in clover (Cooper and Lösel, 1978). This suggested a close relationship between the mycorrhizal colonization rates and the root lipid contents. In addition, the transcript level of lipid transfer protein (LTP) gene, which was believed to be involved in regulation of the intracellular fatty acid pools (Kader, 1996), was increased by the root colonization of *Glomus mosseae* (Blilou et al., 2000). AMF obtained photosynthetically fixed carbon from their host plants mainly in the forms of lipid (triacylglyceride) and carbohydrate (glycogen) (Bago et al., 2003). This elucidated the essential role of the accumulated lipids in mycorrhizal roots during the rice root-fungi symbiosis.

Table 4.4 shows the dissolved organic carbon (DOC) contents in sand
planted with AMF inoculated Hefengzhan and the results were apparently different with those of biomass and root lipid contents in response to different pH levels. Similar with Chapter 3, the highest DOC contents were generally found at pH 8 (p < 0.05) (e.g. BDE-47 (NAMF) at pH 8 = 80.4 mg/kg; BDE-99 (NAMF) at pH 8 = 76.4 mg/kg) (Table 4.4). This finding, explained in details in Chapter 3, could be resulted from the increased negative charges on both organic matter and soil inorganic solid surfaces under higher pH (pH 8) (You et al., 1999). However, this effect of pH levels was diminished after the addition of AMF (Table 4.4). The three AMF generally showed no significant difference in DOC in sand (p > 0.05) (Table 4.4). The elevated DOC content in sand in the treatment with AMF was probably due to the increased root exudates in the rhizosphere by the symbiosis between rice root and AMF (Cardon and Whitbeck, 2007). The significant correlation between the DOC content in sand (BDE-47: r = 0.748, p < 0.01; BDE-99: r = 0.769, p < 0.01) and the root colonization rates further confirmed that this parameter was closely related with colonization of rice roots by AMF.

The increase of mycorrhizal colonization rates of rice roots with the DOC in sand was probably related to the secretion of root exudates (Bécard and Piché, 1989). The root exudates comprise a wide range of organic compounds, such as carbohydrates, amino acids, fatty acids, enzyme etc. (Pinton et al., 2007). It was
found that simultaneous presence of root volatiles (CO₂) and exudates could enhance the fungal growth (G. margarita) by eight times in a clone of root-inducing-T-DNA transformed root of carrot (Bécard and Piché, 1989). A close relationship was also reported between percentage root length infected after 36-day incubation and soluble carbohydrate concentrations within roots after 24-day incubation (Same et al., 1983). It was suggested the net loss of metabolites from the roots (Citrus aurantium L.) enhanced the growth of mycorrhizal fungus (Glomus fasciculatus), in order to allow the AMF improve root phosphorus nutrition (Graham et al., 1981). These explained that the soluble organic carbon was closely associated with the root colonization rates of rice roots.

4.3.2 Contribution of AMF to Dissipation and Degradation of BDE-47 and 99 by Hefengzhan

The interactions between AMF and rice cultivar (Hefengzhan) in dissipation and degradation of BDE-47 and -99 in sand are illustrated in Table 4.5 and Fig. 4.2. There was no BDE-47 and -99 detected in sand in the un-spiked controls. In the unplanted control, there were only 4.07 and 3.97% average losses in the concentrations of BDE-47 and -99 in sand respectively, which could be the result of natural evaporation and/or sorption onto the containers. Compared to the unplanted control, the planted treatments (include both mycorrhizal and
non-mycorrhizal) significantly enhanced the dissipation of BDE-47 and -99 in sand ($p < 0.05$) (Fig. 4.2). The average reduction rates of BDE-47 and -99 in sand in non-mycorrhizal planted treatment (Hefengzhan) were 38.1 and 48.0%, respectively, which were lower than those obtained by Mueller et al. (2006) (around 50 and 62.5% (BDE-47 and -99) in mixed species, with small round red cherriette and yellow gold rush).

There are only a few studies concerning the uptake of BDE-47 and -99 by plants in soil, but none of them focused on the degradation of individual congeners (usually in PBDEs mixture) (Zhao et al., 2012). Therefore, the degradation pathway of individual congeners (in this case of BDE-47 and -99) was difficult to be identified due to the possible synergistic effect and formation of BDE-47 and -99 from debromination of higher brominated PBDEs (e.g. BDE-99 and BDE-209). The dissipation of BDE-47 and -99 in sand varied among different species of AMF but not pH levels (Table 4.5 and Fig. 4.2). This result was different from that mentioned in Section 3.3.4 of Chapter 3, which stated the lowest BDE-209 concentration was observed in the sand planted with Hefengzhan at pH 7, possibly because pH 8 suppressed the growth of rice, but pH 6 did not flavor the reductive debromination of BDE-209 by enzymes (e.g. glutathione-transferase (GST)) which might form negative ions in the process
Table 4.5 Concentrations of BDE-47 and -99 in sand planted with Hefengzhan under the interference of AMF at different pH levels.

<table>
<thead>
<tr>
<th>Treatments</th>
<th>pH 6 (BDE-47)</th>
<th>pH 7 (BDE-47)</th>
<th>pH 8 (BDE-47)</th>
<th>pH 6 (BDE-99)</th>
<th>pH 7 (BDE-99)</th>
<th>pH 8 (BDE-99)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Unplanted</td>
<td>148 A</td>
<td>147 A</td>
<td>148 A</td>
<td>127 A</td>
<td>127 A</td>
<td>126 A</td>
</tr>
<tr>
<td>NAMF</td>
<td>101 A</td>
<td>88.5 A</td>
<td>97.2 A</td>
<td>67.7 A</td>
<td>66.0 A</td>
<td>67.7 A</td>
</tr>
<tr>
<td>GI</td>
<td>71.5 A</td>
<td>69.1 A</td>
<td>81.1 A</td>
<td>57.3 A</td>
<td>53.8 A</td>
<td>56.6 A</td>
</tr>
<tr>
<td>GM</td>
<td>77.8 A</td>
<td>83.5 A</td>
<td>88.5 A</td>
<td>64.4 A</td>
<td>60.8 A</td>
<td>65.4 A</td>
</tr>
<tr>
<td>GV</td>
<td>81.9 A</td>
<td>78.9 A</td>
<td>79.2 A</td>
<td>68.6 A</td>
<td>67.2 A</td>
<td>66.5 A</td>
</tr>
</tbody>
</table>

Unplanted refers to the unplanted control; NAMF refers to the non-mycorrhizal control; GI refers to *Glomus intraradices*; GM refers to *Glomus mosseae*; GV refers to *Glomus versiforme*. Values with the same capital letter are not significantly different among different pHs in the same AMF treatment under one-way ANOVA test (p > 0.05).
Figure 4.2 Dissipation of BDE-47 and -99 in sand planted with Hefengzhan under the interference of AMF at different pHs. (a) Concentration of BDE-47 in sand, (b) BDE-99 in sand. Unplanted refers to the unplanted control; NAMF refers to the non-mycorrhizal control; GI refers to *Glomus intraradices*; GM refers to *Glomus mosseae*; GV refers to *Glomus versiforme*. Bars with the same letter at the top were not significantly different (p > 0.05) in the same pH treatment according to one-way ANOVA test.
(Huang et al., 2013). However, pH 7 did not pose any favorable effect to Hefengzhan, in the degradation of BDE-47 and -99, when compared with the two other studied pH levels (pH 6 and 8) (Table 4.5). This implied that the degradation of BDE-47 and -99 in Hefengzhan (in both mycorrhizal and non-mycorrhizal treatments) was less susceptible to changes of pH than BDE-209.

On the other hand, the highest DOC content (in sand) resulted in pH 8, which did not increase the bioavailability of BDE-47 and -99 to rice plant, through dispersion of soil organic matter adhered with these two congeners (Table 4.4). Therefore, there was no significant correlation between the DOC content and concentrations of BDE-47 and -99 in sand (p > 0.05). The inoculation of rice roots (Hefengzhan) significantly enhanced the dissipation of BDE-47 and -99 in sand when compared with the non-mycorrhizal treatments (p < 0.05) (Fig.4.2). This was further confirmed by the significant correlation between the root colonization rates (Hefengzhan) and BDE-47 and -99 concentrations in sand (BDE-47: r = -0.457, p < 0.05; BDE-99: r = -0.743, p < 0.01). Similar to the findings obtained in Chapters 3, the greatest dissipation rates of BDE-47 and -99 in sand among the three AMF were obtained in GI (BDE-47: 53.1% at pH 7; BDE-99: 57.8% at pH 7). The promoted dissipation of PBDEs in sand might be ascribed to the enhanced plant growth and root exudation, through the symbiosis between AMF and rice
roots (Joner and Leyval, 2003). Nevertheless, the reduction rates of BDE-47 and -99 were lower than that of BDE-209 (63.5%) by Hefengzhan at pH 7 (Table 3.6). This result coincided with those obtained in Mueller et al. (2006), which explained this by the differences of PBDE congeners in physiochemical properties and bioavailability in soil-plant system.

4.3.3 Contribution of AMF to The Uptake and Accumulation of BDE-47 and -99 in Rice

BDE-47 and -99 were taken up and accumulated in roots and shoots of Hefengzhan in both mycorrhizal and non-mycorrhizal treatments after 60-day cultivation, while no PBDEs was detected in the un-spiked controls (Table 4.6, Fig 4.3). Similar patterns of BDE-47 and -99 accumulation in roots and shoots were noted among the three different pH levels (Table 4.6). There was no significant difference in accumulation of BDE-47 and -99 in roots and shoots among various pH levels (p > 0.05), reflecting pH levels did not exert prominent effects on the uptake of BDE-47 by Hefengzhan (Table 4.6). It manifested that their uptake by Hefengzhan was not significantly affected by the alteration of composition of the root exudates and subsequent changes in the rhizospheric activities due to changes of pH (Dakora and Phillips, 2002).

Comparing with the non-mycorrhizal controls, significantly higher
Table 4.6 Concentrations of BDE-47 and -99 in the plant tissues of Hefengzhan under the interference of AMF at different pH levels.

<table>
<thead>
<tr>
<th>Treatments</th>
<th>Roots (nmol/kg)</th>
<th>Shoots (nmol/kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>pH 6</td>
<td>pH 7</td>
</tr>
<tr>
<td>BDE-47 (NAMF)</td>
<td>94.6 A</td>
<td>94.5 A</td>
</tr>
<tr>
<td>BDE-47 (GI)</td>
<td>174 A</td>
<td>172 A</td>
</tr>
<tr>
<td>BDE-47 (GM)</td>
<td>172 A</td>
<td>183 A</td>
</tr>
<tr>
<td>BDE-47 (GV)</td>
<td>133 A</td>
<td>112 A</td>
</tr>
<tr>
<td>BDE-99 (NAMF)</td>
<td>61.2 A</td>
<td>63.7 A</td>
</tr>
<tr>
<td>BDE-99 (GI)</td>
<td>106 A</td>
<td>107 A</td>
</tr>
<tr>
<td>BDE-99 (GM)</td>
<td>111 A</td>
<td>108 A</td>
</tr>
<tr>
<td>BDE-99 (GV)</td>
<td>75.6 A</td>
<td>75.2 A</td>
</tr>
</tbody>
</table>

NAMF refers to the non-mycorrhizal control; GI refers to *Glomus intraradices*; GM refers to *Glomus mosseae*; GV refers to *Glomus versiforme*. Values with the same capital letter are not significantly different among different pHs in the same AMF treatment under one-way ANOVA test (p > 0.05).
Figure 4.3 Accumulation of BDE-47 and -99 in plant tissues (roots and shoots) of Hefengzhan under the interference of AMF at different pHs. (a) Concentration of BDE-47 in roots and (b) in shoots of Hefengzhan; (c) Concentration of BDE-99 in roots and (d) in shoots of Hefengzhan. NAMF refers to the non-mycorrhizal control; GI refers to Glomus intraradices; GM refers to Glomus mosseae; GV refers to Glomus versiforme. Bars with the same letter at the top were not significantly different (p > 0.05) in the same pH treatment according to one-way ANOVA test.
concentrations of BDE-47 and -99 were found in most of the mycorrhizal treatments (p < 0.05) (Fig. 4.3). In both roots and shoots, GI and GM were generally able to significantly promote the uptake of BDE-47 and BDE-99 (p < 0.05), but GV did not do so (p > 0.05) (Fig. 4.3). GI was the most capable AMF interacted with Hefengzhan in the dissipation of BDE-47 and -99 in sand at pH 7 (Fig. 4.2). However, the accumulations of BDE-47 and -99 in roots and shoots in GI treatment were not significantly higher than those of the other two AMF at pH 7 (Fig. 4.3). This might suggest that the dissipation of BDE-47 in sand in the symbiotic treatment between GI and rice roots was not simply by uptake and translocation of rice plants, but also via some extracellular degradation in soil.

There were significant positive correlations between BDE-47 and -99 in plant tissues (roots and shoots) of Hefengzhan and the root colonization rate (BDE-47: r = 0.673, p < 0.01; BDE-99: r = 0.619, p < 0.01), and also the total biomass of plant tissues (roots and shoots) (BDE-47: r = 0.385, p < 0.05; BDE-99: r = 0.560, p < 0.01). These indicated that the accumulation of BDE-47 in Hefengzhan was closely linked with root colonization rate and plant biomass. In addition, the root lipid content of Hefengzhan was also significantly correlated with the concentration of BDE-47 and -99 in rice roots (BDE-47: r = 0.392, p < 0.01; BDE-99: r = 0.417, p < 0.05), coinciding with the results obtained by Huang.
et al. (2010). The concentrations of BDE-47 and -99 in roots were generally higher than in shoots in all treatments (Fig. 4.2). Solutes (e.g. BDE-47 and -99) were translocated upward from roots to shoots and other parts of plants through the xylem, driven by water potential as a result of transpiration (Collins et al., 2006). The solutes in water were then transported laterally into adjacent cells through partition and sorption (Collins et al., 2006). However, organic contaminants (e.g. BDE-47 and -99) were usually retained in root lipid content and prohibited for translocation into shoots (Huang et al., 2010). This demonstrated the importance of root lipid in taking up of PBDEs, a hydrophobic contaminant with high $K_{ow}$.

4.3.4 Contribution of AMF to Debromination of BDE-47 and -99 in Sand and Hefengzhan

The occurrence of debromination and rearrangement of bromide atoms were assumed during the degradation of PBDEs in plants (Wang et al., 2011). The congener profiles of PBDEs in sand, roots and shoots of Hefengzhan are illustrated in Figures 4.4 and 4.5. No distinct pattern could be observed from the profiles among different pH levels (Figures 4.4 and 4.5). In sand, the parental congeners, in this case of BDE-47 and -99, were the two most abundant congeners, respectively in their in the total PBDE congeners profile (Figures 4.4 and 4.5).
Figure 4.4 Degradation profiles of BDE-47 in sand and plant tissues (roots and shoots) of Hefengzhan under the interference of AMF at different pH levels. PBDE congeners profiles in (a) sand, (b) roots and (c) shoots of Hefengzhan. NAMF refers to the non-mycorrhizal control; GI refers to *Glomus intraradices*; GM refers to *Glomus mosseae*; GV refers to *Glomus versiforme*.
Figure 4.5 Degradation profile of BDE-99 in sand and plant tissues (roots and shoots) of Hefengzhan under the interference of AMF at different pH levels. (a) PBDE congeners profile in sand, (b) in roots and (c) in shoots of Hefengzhan. NAMF refers to the non-mycorrhizal control; GI refers to *Glomus intraradices*; GM refers to *Glomus mosseae*; GV refers to *Glomus versiforme*. 
Although inoculation of AMF did not greatly increase the number of PBDE congeners detected in sand, it decreased the percentage of BDE-47 and -99 and promoted the percentage of lower brominated PBDEs (Fig. 4.5a). It could be deduced that the increment of BDE-3 to -28 in proportion to other PBDE congeners was due to the further debromination of BDE-47 resulted from degradation of BDE-99 (Wang et al., 2011). Comparing the PBDE congener profiles in sand and roots (Figures 4.4a and b; 4.5a and b), there were more lower brominated congeners (di- and tri-BDEs for BDE-47; tri- and tetra-BDEs for BDE-99) detected in roots than in sand, implicating the proclivity of BDE-47 and -99 in debromination in the sand-roots system by losing one or two bromide atoms. The percentage of mono-BDE was higher in shoots (dominated at 68.0% at pH 7 in non-mycorrhizal control) than in roots (29.6% at pH 7 in non-mycorrhizal control) in BDE-47 treatments (Fig. 4.4b and c). This result was similar to those presented in BDE-99 (Fig. 4.5b and c), indicating that mono- and di-BDE dominated the congener profile in shoots (sum of mono- and di-BDE in non-mycorrhizal control at pH 7 = 86.1%). It manifested that there might be further debromination through plant metabolism during the translocation of these lower brominated PBDEs from roots to shoots. Generally, there were higher portions of lower brominated PBDE congeners (mono- through tetra-BDEs) in the
mycorrhizal treatments than those in non-mycorrhizal controls in BDE-99 treatments (Fig. 4.5b and c). This observation was more apparent in BDE-99 treatments than BDE-47 treatments (Figures 4.4 and 4.5). The proportion of mono-BDE in shoots with GI inoculation was not significantly higher than the other two AMF in BDE-47 treatment ($p > 0.05$) (Fig. 4.4c), while the proportion of mono-BDE in sand in BDE-99 treatment was obviously higher than that in the other two AMF (Fig. 4.5a).

Wang et al. (2011) also noted BDE-3, BDE-15 and BDE-28 might be the major congeners from debromination of BDE-47. It was also revealed that the debromination mainly occurred at ortho-positions which registered a higher elimination efficiency than those at meta and para positions (Fang et al., 2008; Wang et al., 2011). In the present study, more debromination pathways of BDE-47 in sand were at the ortho position and para position, forming mainly BDE-28 and BDE-3. Nevertheless, when the molecular size increased, the difference in concentrations of the debrominated products from various positions was mitigated (Fang et al., 2008). Consequently, no observable variation in the dominance of debromination positions from BDE-99 could be found.

4.3.5 Partition-limited Model Analysis of Rice Uptake of BDE-47 and -99

A partition-limited model was used for estimating and describing the approach
of the uptake of BDE-47 and -99 by rice plants (Chiou et al., 2001). The quasi-equilibrium factor ($\alpha_{pt}$), which characterised the tendency of a contaminant to equilibrium between the plant and soil at a specific time, could be obtained through calculations from the model (Chiou et al., 2001). Uptake of organic contaminants by plant roots was dependent on the physicochemical properties of the contaminant and the composition of the plant roots (Collins et al., 2006). Therefore, the model could be described as a function of the contaminant partition coefficients, the soil organic matter content in the soil ($f_{som}$), the compositions of plant water ($f_{pw}$) and organic matter ($f_{pom}$), the concentration of a contaminant in specific part of plant (or the whole plant) ($C_{pt}$) and the contaminant concentration in soil ($C_s$) (Section 4.2) (Chiou et al., 2001). Different from the study of BDE-209, only the rice cultivar, Hefengzhan, was employed in the pot trials, so the sorption analysis was conducted with Hefengzhan only (Section 4.2).

In the partition-limited model, the tendency of a particular contaminant to reach equilibrium between plant (or a part of it) and soil at a specific time can be described by quasi-equilibrium factor ($\alpha_{pt}$) (Section 4.2) (Chiou et al., 2001). It may also indicate a ratio of the particular contaminant in plant (parts or a plant whole) to external substrate (Chiou et al., 2001). With the known $\alpha_{pt}$ value of a particular contaminant, the concentrations with respect to the same contaminant in
plant (or a part of it) \( (C_p) \) and soil \( (C_s) \) could be predicted with other known parameters (Chiou et al., 2001). The \( \alpha_{pt} \) values of BDE-47 and -99 between rice roots of Hefengzhan and sand were derived from the fractions of plant water \( (f_{pw} = 0.942) \) and organic matter \( (f_{pom} = 0.0579) \), specific partition coefficients (BDE-47: \( \log K_{ow} = 6.81 \); BDE-99: \( \log K_{ow} = 7.32 \)), and concentrations of the contaminant in the rice roots \( (C_p) \) and sand \( (C_s) \) (Table 4.7) (Section 4.2). The final \( \alpha_{pt} \) values of BDE-47 and -99 in rice roots of Hefengzhan in sand, which were obtained from the parameters mentioned above, were smaller than 1 (BDE-47: \( 1.44 \times 10^{-3} \pm 4.51 \times 10^{-4} \); BDE-99: \( 0.966 \times 10^{-3} \pm 1.41 \times 10^{-4} \)) (Table 4.7), indicating a passive transport dominated uptake approach (Chiou et al., 2001). The \( \alpha_{pt} \) values of BDE-47 \( (1.44 \times 10^{-3}) \) and -99 \( (0.966 \times 10^{-3}) \) in rice roots of Hefengzhan in sand were significantly higher than that of BDE-209 \( (0.115 \times 10^{-3}) \), which might be attributed to the lower \( K_{ow} \) values of BDE-47 and -99 (6.81 and 7.32, respectively) \( (p < 0.05) \) (Table 4.9). This suggested that the movement of BDE-47 and -99 were more readily to reach equilibrium than BDE-209 in sand associated with the same parameters (such as \( f_{pw} \) and \( f_{pom} \)).

4.3.6 Uptake Pathway of BDE-47 and -99 in Rice Roots

The movement of PBDEs in soil water into the root cells can be either through apoplastic or symplastic pathways (Gao et al., 2011). Figure 4.6
Table 4.7 The quasi-equilibrium factors ($\alpha_{pt}$) of different compounds in various plant species and media.

<table>
<thead>
<tr>
<th>Compounds</th>
<th>log $K_{ow}$</th>
<th>Plant species</th>
<th>Medium</th>
<th>$\alpha_{pt}$</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Arazine</td>
<td>2.71</td>
<td>Barley plants</td>
<td>soil (3.5% in SOM)</td>
<td>1</td>
<td>Chiou et al., 2001</td>
</tr>
<tr>
<td>Phenanthrene</td>
<td>4.46</td>
<td>Ryegrass (Lolium multiflorum Lam)</td>
<td>soil (1.5% in SOM)</td>
<td>$8.1 \times 10^{-3}$</td>
<td>Gao et al., 2005</td>
</tr>
<tr>
<td>Pyrene</td>
<td>4.88</td>
<td>Ryegrass (Lolium multiflorum Lam)</td>
<td>soil (1.5% in SOM)</td>
<td>$12 \times 10^{-3}$</td>
<td>Gao et al., 2005</td>
</tr>
<tr>
<td>Hexachlorobenzene</td>
<td>5.5</td>
<td>Barley plants</td>
<td>soil (3.5% in SOM)</td>
<td>0.2</td>
<td>Chiou et al., 2001</td>
</tr>
<tr>
<td>2,4,6,2',4'-polychlorinated biphenyl (PCB)</td>
<td>5.92</td>
<td>Barley plants</td>
<td>soil (3.5% in SOM)</td>
<td>0.19</td>
<td>Chiou et al., 2001</td>
</tr>
<tr>
<td>Dichlorodiphenyltrichloroethane (DDT)</td>
<td>6.36</td>
<td>Barley plants</td>
<td>soil (3.5% in SOM)</td>
<td>0.11</td>
<td>Chiou et al., 2001</td>
</tr>
<tr>
<td>2,2',4,4'-Tetrabromodiphenyl ether (BDE-47)</td>
<td>6.81</td>
<td>Rice-Hefeng zhan (Oryza sativa)</td>
<td>soil (0.88% in SOM)</td>
<td>$1.44 \times 10^{-3}$</td>
<td>ATSDR, 2004b; This study</td>
</tr>
<tr>
<td>2,2',4,4',5-Pentabromodiphenyl ether (BDE-99)</td>
<td>7.32</td>
<td>Rice-Hefeng zhan (Oryza sativa)</td>
<td>soil (0.88% in SOM)</td>
<td>$0.966 \times 10^{-3}$</td>
<td>ATSDR, 2004b; This study</td>
</tr>
<tr>
<td>Decabromodiphenyl ether (BDE-209)</td>
<td>9.97</td>
<td>Rice-Hefeng zhan (Oryza sativa)</td>
<td>soil (0.88% in SOM)</td>
<td>$0.115 \times 10^{-3}$</td>
<td>ATSDR, 2004b; This study (Chapter 3)</td>
</tr>
</tbody>
</table>
illustrates the concentration-dependent uptake curves of BDE-47 and -99 in the fresh and dead roots of Hefengzhan. The concentrations of BDE-47 and -99 in dead rice roots were demonstrated to be linearly related to the concentration of BDE-99 in the external solutions, while the uptake isotherms of the contaminants by living fresh roots was in non-linear shape (Fig. 4.6). It was shown that the uptake coefficient (the ratio of PBDEs concentrations in roots to that in external solutions) of BDE-47 and -99 in dead roots was higher than those in fresh roots (Fig. 4.6), revealing an apoplastic pathway which dominated the uptake of BDE-47 and -99 into the rice roots (Su and Zhu, 2007).

Wild et al. (2005) applied the two-photon excitation microscopy (TPEM) in the manifestation of PAHs uptake by maize and wheat roots, and showed that it was dominated by the apoplastic pathway. Su and Zhu (2007) carried out another study concerning the uptake behaviour of PAHs (PHN and PYR) in dead and fresh roots of rice (China rice Jiahua-1) respectively, and observed that generally more PAHs were partitioned into the dead roots than the fresh roots in the same concentration of external solution. The results were similar to the present study (Fig. 4.6), confirming the uptake of BDE-47 and -99 in rice roots of Hefengzhan was dominated by the apoplastic pathway. Active transport of PBDEs into the rice roots was not possible after root cells were killed by heat, and thus
Figure 4.6 Concentration-dependent uptake curves of BDE-47 and -99 with fresh and dead rice roots of Hefengzhan. Error bars denote the standard errors of the means (n=3).
BDE-47 and -99 was probably move passively down the concentration gradient into the root cells through cell wall (apoplastic pathway). When active transported was prohibited in dead roots, the concentrations of BDE-47 and -99 still increased proportionally with that in the external solution. It implicated that the uptake process of BDE-47 and -99 was predominated by partitioning into lipophilic components of rice roots, such as root cell walls (Chen et al., 2009). Root cell walls had more aromatic carbon and lower polarity than the bulk roots, and thus phenanthrene possessed a higher sorption capacity in root cell walls rather than in bulk roots (Chen et al., 2009). Therefore, BDE-47 and 99 were more likely retained in root cell walls rather than moving across cell membrane and cytoplasm with the symplastic water stream due to its high lipophilicity (log $K_{ow} = 7.32$). Therefore, the higher uptake coefficients (the ratio of PBDEs concentrations in roots to that in external solutions) of BDE-47 and -99 in dead roots than those in fresh roots (Fig. 4.6), indicated a passive pathway dominated their uptake by roots, and apoplastic pathway was presumably the approach that BDE-47 and -99 in aqueous solutions enter plant root cells (Su and Zhu, 2007). In the fresh roots, there was a slight decrease in uptake coefficients when the concentration of BDE-47 and -99 in external solutions (Fig. 4.6). This might be due to the uptake retardation or metabolism of the contaminants in rice roots. The patterns of the
sorption curves of BDE-47 and -99 in dead and fresh roots were similar to those obtained in BDE-209, indicating the uptake pathways of these contaminants into root cells were similar and all in apoplastic approach.

4.4 Conclusion

The rice cultivar, Hefengzhan, was able to take up and accumulate BDE-47 and -99. Spiking of BDE-47 and -99 (75 μg/L) did not affect plant growth, in respect of root and shoot mass, and also root lipid content. AMF exerted a prominent effect in enhancing plant growth, dissipation of BDE-47 and -99 in sand, and accumulation of BDE-47 and -99 in plant tissues (roots and shoots). The reduction rates of the studied PBDEs in soil-plant system in ascending order were BDE-47 > BDE-99 > BDE-209 in the present study, and agreed with the hypothesis. BDE-47 was more readily to reach equilibrium than BDE-99 and -209 in rice roots and sand. The uptake of BDE-47 and -99 into root cells was mainly by apoplastic pathway, which was similar to those observed in BDE-209.
CHAPTER 5
TREATMENT OF DECABROMODIPHENYL ETHER (BDE-209) USING COMBINED TiO$_2$ PHOTOCATALYSIS AND CONSTRUCTED WETLAND SYSTEM

5.1 Introduction

The abilities of nano-sized TiO$_2$ and rice plants (associated with AMF) in promoting degradation of PBDEs have been demonstrated in the previous chapters (Chapters 2 to 4). Both TiO$_2$ photocatalysis and phytoremediation are promising technologies in treating aquatic organic contaminants, including PBDEs (Imfeld et al., 2009; Huang et al., 2013). Aqueous TiO$_2$ suspension was applied in the debromination of BDE-209 and the degradation efficiency reached 95.6% after 12-h illumination (Huang et al., 2013). The debromination percentage of BDE-209 was also reported as high as 56.4% by AMF (Glomus mosseae) inoculated ryegrass (Wang et al., 2011). Nevertheless, TiO$_2$ photocatalysis and phytoremediation have their respective drawbacks and limitations in practical application, which include the high cost and environmental concerns about the by-products (Table 5.1). Most of the sewage treatment facilities are not specifically designed for treating emerging contaminants, including PBDEs.
Table 5.1 Limitations of TiO\textsubscript{2} photocatalysis and phytoremediation in treatment of contaminants.

<table>
<thead>
<tr>
<th>Contaminants treatment technologies</th>
<th>Limitations</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>TiO\textsubscript{2} photocatalysis</td>
<td>• the operation cost and energy consumption are high</td>
<td>Comninellis et al., 2008</td>
</tr>
<tr>
<td></td>
<td>• may generate by-products and/or metabolites more toxic than the parental compounds</td>
<td>Sun et al., 2009</td>
</tr>
<tr>
<td></td>
<td>• require additional separation process of TiO\textsubscript{2} from water after treatment</td>
<td>Thiruvenkatachari et al., 2008</td>
</tr>
<tr>
<td></td>
<td>• may generate by-products and/or metabolites more toxic than the parental compounds</td>
<td>Alkorta and Garbisu, 2001</td>
</tr>
<tr>
<td>Phytoremediation</td>
<td>• relatively slow process</td>
<td>Cunningham, 1995</td>
</tr>
<tr>
<td></td>
<td>• the level of contaminants must be within the limit of toxic tolerance of the plants</td>
<td>Cunningham, 1995</td>
</tr>
<tr>
<td></td>
<td>• efficiency depends on bioavailability of contaminant</td>
<td>Pilon-Smits, 2005</td>
</tr>
</tbody>
</table>
(Bolon et al., 2009). The undegraded or partially degraded PBDEs might be discharged into the environment together with the wastewater. Approximately 2280 kg/year of PBDEs were released through wastewater discharge in Pearl River Delta in 2006 (Peng et al., 2009). In addition, most of the BDE-209 in modern wastewater treatment works is not completely degraded but sorbed in the sludge (North, 2004). Therefore, there is an urgent need to develop a more environmentally friendly and efficient treatment technology for abating the considerable amount of PBDEs contained in sewage.

In order to complement the respective limitations of TiO$_2$ photocatalysis and phytoremediation, a combined system involving these two technologies was proposed in the present study. The synergistic effect of the combined system has been demonstrated, in the treatments of some organic contaminants, such as 4-nitrophenol (4NP) and TOC (Herrera-Melián et al., 2012; Antoniadis et al., 2007). There is a lack of such information about the treatment of PBDEs in sewage using this combined system, and therefore studies related to treatments of these organic compounds (e.g. 4-nitrophenol) would provide some essential information concerning the feasibility of treating PBDEs by this innovative technology. Herrera-Melián et al. (2012) indicated that the combined system involved solar TiO$_2$-photocatalysis with CWs (*Phragmites* sp., *Cyperus* sp.,
*Pontederia* sp., and *Scirpus* sp.) had resulted in 100% removal of 4NP and 49.5% removal of TOC for the initial 4NP concentration at 289 mg/L. More importantly, the biodegradability of the organic contaminant (4NP) was enhanced and the removal and detoxification rates were promoted (from 4.11 mg/L/h to 6.81 mg/L/h) after the combination of these two technologies (Herrera-Melián et al., 2012). Antoniadis et al. (2007) also reported that the organic load (DOC (mg/L)) was diminished by 98.6% in real cesspool wastewater after treating in the combined system under solar irradiation.

In this investigation, the combined system involved two wetland plant species, *Oryza sativa* (rice) and *Phragmites australis* (common reed). The ability of rice in degradation and accumulation of PBDEs has already been investigated previously (Chapters 3 and 4). Common reed, one of the most widely distributed plant species in the world and a promising wetland plant commonly used in constructed wetland for wastewater treatment (Struyf et al., 2007; Dunbabin and Bowmer, 1992), was included for the combined system. Oxygen release from roots of aquatic plants in constructed wetlands is important for aerobic degradation of oxygen-consuming substances and nitrification (Brix, 1994). It was estimated that *Phragmites* roots could release oxygen up to 12 g/m$^2$/day (Armstrong et al., 1990), which may help in degradation of some rhizospheric
contaminants, such as pyrene (Jouanneau et al., 2005). In addition, reed is a productive plant and could accumulate considerable amounts of nutrients (nitrogen: > 2 t/ha/y; phosphorus: > 100 kg/ha/y) in the biomass (Brix, 1994). To date, there is no published article concerning the removal of BDE-209 by reed, but it was reported that reed could remove DDT and PCB from spiked solution and accumulate them in plant tissues (root, stem and leaf) (Chu et al., 2006a). This suggested the feasibility of using this wetland plant in remediation of persistent organic pollutants in wastewater treatment.

The previous chapters (Chapters 2 to 4) identified the optimal operating conditions of TiO$_2$ photocatalysis (visible light: pH 12, 5-20 mg/L humic acid, anatase/rutile TiO$_2$) and phytoremediation (rice cultivars: Hefengzhan, pH 7, symbiosis with Glomus intraradices) of PBDEs. Based on these results, a combined photocatalysis (TiO$_2$ and visible light) and constructed wetland system (Oryza sativa (Hefengzhan) and Phragmites australis (common reed) was set up for enhancing the degradation efficiency of PBDEs in water. Comparison will be made between the PBDEs removal efficiencies of the combined system and those of individual systems (TiO$_2$ photocatalysis and phytoremediation by rice and reed plants) alone. It was hypothesized that the removal efficiencies of BDE-209 (the maternal congener) might be higher in the combined system than those in the
individual systems due to the enhanced biodegradability of PBDEs in photocatalysis (Herrera-Melián et al., 2012).

The major objectives of this experiment were to (1) investigate the removal efficiency of PBDEs treatment in artificial BDE-209 spiked wastewater; (2) compare the performance of the system with those of the individual systems alone, in respect of PBDEs removal; (3) explore the fate of BDE-209 from the TiO$_2$ photocatalysis compartment to the constructed wetland; (4) examine the differences between Hefengzhan and common reed in taking up PBDEs, in order to identify the feasibility of treating PBDEs in sewage using this combined system. It was hoped to provide a more efficient and environmental friendly alternative for the modern wastewater treatment facility.

5.2 Materials and Methods

5.2.1 TiO$_2$ Photocatalysis/Constructed Wetland System

Artificial wastewater was produced by diluting the aqueous BDE-209 (Dr. Ehrenstorfer (Germany)) stock in DMSO (Sigma-Aldrich, USA) with deionized water to the final concentrations: 75 and 750 µg/L (Chen et al., 2012; Machate et al., 1997). According to the results obtained in Chapter 2, the wastewater was added with 5 mg/L humic acid. Although the previous results stated that the reduction rate of BDE-209 was the highest at pH 12, the wastewater was adjusted
to pH 7 for better growth of plants. The combined treatment system comprised two separate phases: (1) TiO$_2$ photocatalysis and (2) constructed wetlands (24 tanks). In the first phase, the BDE-209 spiked synthetic wastewater was pre-treated in a laboratory-scale photocatalytic reactor, and followed by discharging into constructed wetland tank in the greenhouse of Hong Kong Baptist University.

Photocatalysis of BDE-209 was conducted in 1% aqueous suspension of nano-sized TiO$_2$ catalyst (nanopowder mixture of anatase (65%) and rutile (35%) crystalline forms, < 100 nm particle size, Sigma-Aldrich (USA)), adjusted to pH 7 under visible light (400–530 nm). The mixture suspension was placed in a fluorescent lamp chamber (103 μmol/m$^2$/sec) and continuously stirred for 3 days, which was the half-life (3.05 d) of BDE-209 in 1% aqueous suspension of nano-sized TiO$_2$ catalyst (Chapter 2). The residual PBDEs in the solution were extracted by liquid-liquid extraction and quantified by a gas chromatography-mass spectrometry (GC-MS) after the photocatalytic reaction (US EPA, 1996a). The partially degraded wastewater was centrifuged at 3000 rpm and passed through 0.2-μm PTFE membrane filter to retain the TiO$_2$ particles (Advantec MFS, Inc., Japan). The filtered solution was discharged as inflow for the constructed wetlands, which was the second phase of the treatment.
The second phase of PBDEs treatment was conducted in sub-surface flow constructed wetlands (Fig. 5.1), set up in the greenhouse for each treatment (include both BDE-209 spiked (75 and 750 µg/L), non-spiked wastewater and unplanted controls) for six months. There were three constructed wetland replicates, randomly located on the benches inside the greenhouse. The dimension of each constructed wetland was 60 cm (L) × 30 cm (W) × 60 cm (H) with 1° slope (US EPA, 1988). Each constructed wetland was divided into three zones: inlet (15 cm), treatment (30 cm) and outlet (15 cm). The inlet and outlet zones were filled up with gravels (around 2.5 cm in diameter), while the treatment zone in between contained the soil collected from Sai Keng, Hong Kong SAR. The constructed wetlands were acclimatized for one month, so as to let the seedlings adapt to the set-up, before filling with PBDEs wastewater (Wu et al., 2008). The photocatalytically pre-treated PBDEs wastewater was discharged into the constructed wetland after the acclimatization. The water level was kept at 10 cm above the soil surface. The hydraulic residence time (HRT) of each wetland was 6 days with a hydraulic flow rate, of 1.35 L per day (Antoniadis et al. 2007).

5.2.2 Preparation of Soil

Clean surface sand soil without detectable PBDEs was collected from Sai Keng (22°25’N, 114°16’E) (Tam and Wong, 1998), on the eastern side of Hong
Figure 5.1a Schematic diagram of the sub-surface flow constructed wetland (front side)
Figure 5.1b Schematic diagram of the sub-surface flow constructed wetland (lateral side)
Kong. The soil was soaked with deionized water to lower the salinity (EC = 312 ± 24.0 μS/cm) which was suitable for rice plant growth (Scardaci et al., 2002). The soil was air-dried for two weeks, ground and passed through a 2 mm sieve to remove coarse stones and plant tissues. The soil was autoclaved at 121 °C for 2 h for sterilization before use. The soil was analyzed for basic physicochemical properties and the results are listed in Table 5.2 (Allen, 1989). The soil was adjusted to pH 7 with 0.1 M NaOH before use. Arbuscular mycorrhizal fungi, *Glomus intraradices* (International Culture Collection of (Vesicular) Arbuscular Mycorrhizal Fungi (INVAM), France) were added to the autoclaved soil in the ratio of 1.5 kg soil to 50 g inoculum (Li et al., 2011).

5.2.3 Selection of Rice Cultivars and AMF Species

Hefengzhan and *Glomus intraradices* (GI) were chosen for the combined system to treat BDE-209 because Hefengzhan showed the best performance in uptake and degradation of PBDEs, in the interaction with GI (Chapters 3 and 4).

5.2.4 Collection of Seeds and Preparation of Seedlings

Rice (*Oryza sativa* L., Hefengzhan) seeds were obtained from Guangdong Academy of Agricultural Sciences, China, whilst the reed (*Phragmites australis* L.) seeds were collected from Mai Po World Wildlife Fund Reserve, Hong Kong in the seeding season. The seeds were surface sterilized in a 10% H₂O₂ (wt/wt)
solution for 15 min, followed by thoroughly rinsing with sterilized deionized water.

The sterile reed seeds were cold shocked at 4 °C for 3 days before germination in moist sterile sand at 25 °C in the growth chamber (Sanyo MRL-351H, Japan) (Oliveira et al., 2001). The reed seedlings were watered regularly with deionized water and supplemented with 20% Hoagland’s nutrient solution (1.0 mM Ca(NO$_3$)$_2$, 1.0 mM KNO$_3$, 0.4 mM MgSO$_4$, 0.2 mM KH$_2$PO$_4$, 10 μM Fe(II)-EDTA, 9 μM H$_3$BO$_4$, 0.2 μM ZnSO$_4$, 0.1 μM CuSO$_4$, 2 μM MnSO$_4$, 0.02 μM (NH$_4$)$_6$Mo$_7$O$_24$) (Hoagland and Arnon, 1938) once a week. The sterile rice seeds were placed on sterilized nylon mesh for germination in the dark in the growth chamber (Sanyo MRL-351H, Japan). The rice seedlings were then transferred to 20% Hoagland’s nutrient solution at day 7 after germination. Seedlings of a similar size (around 15 cm in length) were selected for the constructed wetland experiments. Non-spiked soil with plant and spiked soil without plant (controls) were set up. Five seedlings were transplanted into every constructed wetland tank (Li et al., 2011). The plants were acclimatized for 1 month in the glass wetland tanks before treating with wastewater (Wu et al., 2008).

5.2.5 Pot Experiments with Reed Plants

Reed seedlings with similar size (around 30 cm in length) were selected for
the pot experiments and cultivated for 90 days. Non-spiked soil (from Sai Keng) with plants and spiked soil without plants (sowed as controls) were set up. Two reed seedlings were transplanted into each plant treatment pot. The upper top-soil 5-6 cm of each pot was covered with non-spiked sterilized soil to establish a buffer layer to reduce the loss of BDE-209 due to evaporation and photolysis (Wang et al., 2011). The positions of the pots (placed in a greenhouse at Hong Kong Baptist University with temperature control, 28/23 °C day/night) were re-randomized every 3 days and all the treatments were conducted in triplicate. All the pots were watered with deionized water every day and conditioned with 20% Hoagland’s nutrient solution once a week (Hoagland and Arnon, 1938).

5.2.6 Harvesting and Sample Collection

Wastewater samples (100 mL) were collected at the outlet at 6-day intervals, followed by filtering with 550 °C heated glass fibre filter paper (Whatman GF/A, UK) to remove suspended particles. The collected wastewater was extracted by liquid-liquid extraction for PBDEs analysis immediately (US EPA, 1996a). After 5 months, the plant shoots, roots and rice grains were harvested separately, with roots carefully removed from the soil. Shoot and root samples were rinsed separately three times with D.I. water, and blotted with tissue papers. The soil in the rhizospheric soil was collected. A portion of the freshly collected roots (about
2 g) was reserved for the analysis of AMF colonization rate. The rest of the plant tissues and soil samples was frozen at −20 °C overnight, and then the plant tissues and soil samples were freeze-dried for 3 d and 7 d, respectively. After weighing the dry weight of plant tissues, the dried shoot, root, rice grains and soil samples were ground separately and stored at −20 °C before chemical analyses (US EPA, 2007).

5.2.7 Quantification of Root Colonization

The freshly collected root samples were washed with D.I. water 3 times to remove any adhering sand and then put into 10% (w/v) KOH solution. The solution containing the roots was heated at 90 °C for 40 min. The KOH solution was then discarded and the roots were washed with D.I. water 3 times. The root tissues were then bleached with fresh alkaline H₂O₂ solution for 15 min (30 mL 10% H₂O₂ + 3 ml of NH₄OH + 567 mL D.I. water). The root tissues were again washed three times with D.I. water. The samples were acidified by immersing in 1% HCl for 3-4 min, followed by staining with 0.05% lactophenol blue in 90 °C water bath for 15 min. The roots were then destained by immersing in D.I. water and examined for fungal colonization under a microscope (Philips and Hayman, 1970). A drop of glycerol and lactic acid solution (1:1 = v:v) was added onto twenty segments of stained roots (each approximately 1 cm long) placed on a
glass slide and then covered with a cover slip. There were three replicates for each subsample. The length of infected cortex was recorded under microscopic assessment and calculated as a percentage of colonization (Giovannetti and Mosse, 1980).

5.2.8 Lipid Extraction

Total lipid in roots was extracted by mixing chloroform–methanol (1:2 v/v) with the samples according to Bligh and Dyer (1959). The lipid fraction was recovered from the solvent mixture after shaking for 5 min and the solvent mixture with extracted lipid was concentrated using a rotary evaporator. The weight of the extracted lipid of each sample was measured for the calculation of total root lipid content (Lee et al., 2010).

5.2.9 Dissolved Organic Carbon in Soil

The DOC of fresh soil samples was extracted with deionized water (soil: water = 1:5). The extract was then analyzed with a total organic carbon (TOC) analyzer (Shimadzu TOC-Vcph, Japan).

5.2.10 Extraction of PBDEs

The extractions of PBDEs in plant and soil were based on Standard Method 1614 and 3540C respectively (US EPA, 2007, US EPA, 1996b). Ten grams of soil sample were spiked or two to five grams of plant tissues with 100 µL of 200 µg/L
mass-labelled ($^{13}$C$_{12}$) solution/mixture (MBDE-MXE) (Wellington Laboratories Inc, Canada) for extraction recoveries of PBDEs. The samples were placed in a thimble with 5 g anhydrous sodium sulphate (US EPA, 1996b). The samples were then extracted with 90 mL acetone (pesticide grade, Tedia), dichloromethane (DCM) (pesticide grade, Tedia) and n-hexane mixture (1:1:1, v:v:v) in a 150-mL round-bottomed flask connected with Soxhlet apparatus for 18 h. The extract was allowed to cool after the extraction was completed. The extract was then concentrated to around 10 mL by a rotary evaporator, followed by cleaning up using a standard clean-up method 3620B (US EPA, 1996c). In summary, extracts were eluted with n-hexane (pesticide grade, Tedia) in a florisil packed glass column. After the clean-up, the extracts were reduced to about 1 mL by a rotary evaporator and evaporated by a gentle stream of nitrogen gas until the volume reached 100 μL. Then, $^{13}$C$_{12}$-BDE-138 (200 ng/mL) was added to the sample before GC injection as the injection standard.

5.2.11 Chemical Analysis of PBDEs

GC-MS analysis was carried out on an Agilent 7890A GC-MS instrument connected with an Agilent 5975C inert Mass Selective Detector triple-axis detector (Agilent Technologies, USA) and a 15 m x 0.25 mm x 0.25 μm capillary column (Agilent technologies). Concentrations of 30 PBDEs (3, 7, 15, 17, 28, 47,
49, 66, 71, 77, 85, 99, 100, 119, 126, 138, 153, 154, 156, 183, 184, 191, 196, 197, 206, 207, 209) were determined based on the Standard Method 1614 (US EPA, 2007). The temperature program of the GC oven was: temperature held at 100 °C for 5 min, increased at 10 °C/min to 310 °C, and then held for 20 min. A 1 μL of each sample was injected into the GC column.

5.2.12 Quality Control

Extraction and analysis were conducted in the dark, in order to minimize exposure to light. A procedure blank was included in each batch of extraction. The LOD which was defined as three times the standard deviation in the blank samples: it ranged from 0.5 ng/g (BDE-85) to 4 ng/g (BDE-206). The LOD of BDE-209 was 95 ng/g. A mass-labeled polybrominated diphenyl ethers (\(^{13}\)C\(_{12}\)-PBDEs) (PBDEs 3, 15, 28, 47, 99, 153, 154, 183, 197, 207 and 209) (40 ng/mL) were added into samples prior to extraction and the recoveries ranged from 73-107 %.

5.2.13 Data Analysis

The statistical analyses of the data were performed by using SPSS version 16 software package for Windows. The statistical values were calculated for all triplicates. Normality of the data was checked by the Shapiro-Wilk test before conducting all further calculations. A 95% confidence limit (p < 0.05) was applied
for the indication of any significant differences between samples.

5.3 Results and Discussion

5.3.1 Basic Physicochemical Properties of the Soil

Summary physicochemical properties of the soil are listed in Table 5.2. The soil collected from a local mangrove swamp contained mostly sand in texture (96.2%) (Table 5.2). The soil organic matter of the sand soil (3.36%) was slightly higher than that of the sand used in Chapter 3 (0.878%). The original pH of soil was 6.45 ± 0.160, but it was calibrated to 7.03 ± 0.0258 with 0.1 M NaOH before use (Table 5.2).

5.3.2 TiO$_2$ Photocatalysis of BDE-209

The results from TiO$_2$ photocatalysis of BDE-209 are illustrated in Fig. 5.2. Comparing the concentrations of BDE-209 in water in the dark treatment after 3 days with the initial concentrations (75 and 750 μg/L), there were 9.83 and 7.75% losses of BDE-209 in the 75 and 750 μg/L solutions, respectively. The loss was believed to be mainly attributed to the adsorption on the container and evaporation of BDE-209. The residual concentration of BDE-209 in the light treatment was significantly lower than that of the dark treatment (p < 0.05) (Fig. 5.2), which explained the photolysis property of BDE-209 (Söderström et al., 2004). The concentrations of BDE-209 in the light treatment were also significantly lower.
### Table 5.2 Basic physicochemical properties of soil collected from Sai Keng

<table>
<thead>
<tr>
<th>Soil properties</th>
<th>Sai Keng soil</th>
</tr>
</thead>
<tbody>
<tr>
<td>Soil texture (%)</td>
<td>Clay: 2.68 ± 0.0837; Silt: 1.13 ± 0.0858; Sand: 96.2 ± 0.00321</td>
</tr>
<tr>
<td>pH</td>
<td>7.03 ± 0.0258</td>
</tr>
<tr>
<td>EC (μS/cm)</td>
<td>312 ± 24.0</td>
</tr>
<tr>
<td>Total organic matter (%)</td>
<td>3.36 ± 0.138</td>
</tr>
<tr>
<td>Total nitrogen (TN) (%)</td>
<td>0.0304 ± 0.006</td>
</tr>
<tr>
<td>Total phosphorus (TP) (%)</td>
<td>0.0225 ± 0.003</td>
</tr>
<tr>
<td>Nitrate-N (NO$_3^-$–N) (μg/g dry weight)</td>
<td>9.82 ± 1.77</td>
</tr>
<tr>
<td>Inorganic phosphate (PO$_4^{3-}$–P) (μg/g dry weight)</td>
<td>3.32 ± 0.769</td>
</tr>
</tbody>
</table>
Figure 5.2 Photocatalysis of BDE-209 in aqueous TiO$_2$ solution. Bars with the same letter at the top were not significantly different (p > 0.05) according to one way ANOVA test.
than that in the TiO$_2$/light treatment (p < 0.05) (Fig. 5.2), which confirmed the
catalytic property of HA sensitized TiO$_2$ on degradation of BDE-209 under visible
light (Zhao et al., 2004). The TiO$_2$ photocatalysis of BDE-209 spiked wastewater
led to 56.3% (34.2 nmol/L) and 54.7% (354 nmol/L) average loss of BDE-209
(initial concentrations of 75 and 750 μg/L respectively), before discharging into
the constructed wetland.

5.3.3 Phytoremediation of BDE-209 by Common Reed (*Phragmites australis*)

The ability of *Oryza sativa* (rice) associated with AMF (GI) to remediate
BDE-209-contaminated sand has been presented in Chapter 3, so only the reed
plant was used in the phytoremediation study of BDE-209 in this experiment. The
results will be compared to the results generated from the combined system. The
ability of common reed associated with AMF (*Glomus intraradices* (GI)) in
taking up and degradating BDE-209 in soil has been investigated and the results
are shown in Tables 5.3 and 5.4. AMF (GI) did not significantly promote the
biomass of roots and shoots of reed (p > 0.05), and spiking of BDE-209 did not
show any significant change in the biomass of reed (p > 0.05) (Table 5.3).
However, Dolinar and Gaberščik (2010) found significant correlations between
the abundance of mycorrhizal colonization and the dry weight, plant height and
leaf area of *Phragmites australis*. The enhancement of dry weight by mycorrhizal
Table 5.3 Biomass of reed associated with AMF (GI) in BDE-209 spiked soil

<table>
<thead>
<tr>
<th>Treatment</th>
<th>AM Fungi</th>
<th>Root (g/pot)</th>
<th>Shoot (g/pot)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>NAMF</td>
<td>2.46 ± 0.319 a</td>
<td>4.09 ± 0.298 a</td>
</tr>
<tr>
<td>Control</td>
<td>GI</td>
<td>2.95 ± 0.740 a</td>
<td>5.28 ± 1.30 a</td>
</tr>
<tr>
<td>BDE-209</td>
<td>NAMF</td>
<td>2.62 ± 0.571 a</td>
<td>3.30 ± 0.639 a</td>
</tr>
<tr>
<td>BDE-209</td>
<td>GI</td>
<td>2.72 ± 1.15 a</td>
<td>4.51 ± 1.13 a</td>
</tr>
</tbody>
</table>

Control refers to non-spiked control; BDE-209 refers to BDE-209 spiked treatment; NAMF refers to non-mycorrhizal treatment; GI refers to *Glomus intraradices* treatment. Values in the same column with the same letter are not significantly different ($p > 0.05$) according to one way ANOVA test.
Table 5.4 Concentrations of BDE-209 in soil and uptake of BDE-209 by reed associated with AMF (GI)

<table>
<thead>
<tr>
<th>Treatments</th>
<th>NAMF (nmol/kg)</th>
<th>GI (nmol/kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Unplanted soil</td>
<td>74.6 ± 5.08 a</td>
<td>73.3 ± 10.8 a</td>
</tr>
<tr>
<td>Planted Soil</td>
<td>40.2 ± 8.32 b</td>
<td>37.4 ± 4.76 b</td>
</tr>
<tr>
<td>Roots</td>
<td>12.4 ± 1.02 c</td>
<td>13.9 ± 1.30 c</td>
</tr>
<tr>
<td>Shoots</td>
<td>2.11 ± 0.891 d</td>
<td>1.84 ± 0.498 d</td>
</tr>
</tbody>
</table>

NAMF refers to non-mycorrhizal treatment, and GI refers to *Glomus intraradices* treatment. Values in the same column with the same letter are not significantly different among treatments (p > 0.05) according to one way ANOVA test.
colonization was the result of promoted nutrient uptake (Smith and Read, 2008).

In this study, the non-significant difference between the biomass of roots and shoots in mycorrhizal and non-mycorrhizal treatments was probably due to the low infection rate of GI in reed roots (4.00% on average).

There were only 4.58 and 6.23% losses (probably from adsorption to container) of BDE-209 in the unplanted soil due to the presence of buffer soil covered over the contaminated soil for preventing evaporation. The concentration of BDE-209 in planted soil was significantly lower than that in unplanted soil (p < 0.05), suggesting the ability of common reed in promoting the degradation of BDE-209 in soil (Table 5.4). There were 48.1 ± 10.4 and 46.0 ± 10.8% losses of BDE-209 in soil planted with common reed with and without AMF (GI), respectively. Inoculation of reed roots with GI (4.13%) did not significantly increase the BDE-209 degradation in soil and accumulation in plant tissues (roots and shoots) (p > 0.05) (Table 5.4). This result contradicted with those from Chapters 3 and 4, which stated significant correlations between the AMF colonization rates and PBDEs degradation in the growth substrate (sand) of rice. Root BDE-209 concentrations in both the non-mycorrhizal control and GI treatment were prominently greater than those in shoots (p < 0.05) (Table 5.4). This gives a translocation factor (C_{shoot}/C_{root}) < 1 and a suggestion that inclination
BDE-209 is retained in roots rather than in shoots of reed (Huang et al., 20110). Chu et al. (2006a) investigated the accumulation, distribution and transformation of o,p’-DDT, p,p’-DDT and PCBs by reed and rice plants (*Oryza sativa* L. subsp. *indica*, cv. Wuyujin 3). It was found that highly lipophilic contaminants, such as DDT and PCBs, may be accumulated in reed by both passive adsorption and active absorption, but with passive adsorption the first process on root surfaces (Chu et al., 2006a).

5.3.4 Combined Photocatalysis/Wetland System

5.3.4.1 Effects on Plant Growth in the Combined System

The BDE-209 wastewater was photocatalytically pre-treated with TiO$_2$ aqueous suspension for 3 days, and followed by plant-mediated treatment. The effects of the pre-treated wastewater on the growth of rice and reed plants are demonstrated in Tables 5.5 and 5.6. The average root colonization rates of rice (5.81%) and reed (3.27%) were very low in the flooded constructed wetland. Oliveira et al. (2001) explained that the low root colonization rates of reed (< 5%) were probably because the time for roots submerged in water was too long (7 months). The increase of the initial concentrations of BDE-209 in the synthetic wastewater did not exert any significant changes on the biomass of rice plants (root, shoot and rice grain) and reed (root and shoot) (p > 0.05) (Tables 5.5 and
Table 5.5 The biomass of rice plants grown in the constructed wetlands fed with photocatalytically pre-treated wastewaters (with different initial BDE-209 concentrations)

<table>
<thead>
<tr>
<th>Initial BDE-209 concentrations</th>
<th>Biomass (g/tank)</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Root</td>
<td>Shoot</td>
<td>Rice grains</td>
</tr>
<tr>
<td>0 µg/L (control)</td>
<td>7.94 ± 1.73 a</td>
<td>21.2 ± 3.87 a</td>
<td>4.01 ± 0.881 a</td>
</tr>
<tr>
<td>75 µg/L</td>
<td>7.62 ± 1.52 a</td>
<td>18.7 ± 2.15 a</td>
<td>2.99 ± 1.31 a</td>
</tr>
<tr>
<td>750 µg/L</td>
<td>8.21 ± 2.70 a</td>
<td>16.1 ± 2.79 a</td>
<td>4.71 ± 1.75 a</td>
</tr>
</tbody>
</table>

Values in the same column with the same letter are not significantly different among treatments (p > 0.05) according to one way ANOVA test.
Table 5.6 The biomass of reed plants grown in the constructed wetlands fed up with photocatalytically pre-treated wastewaters (with different initial BDE-209 concentrations)

<table>
<thead>
<tr>
<th>Initial BDE-209 concentrations</th>
<th>Biomass (g/pot)</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Root</td>
<td>Shoot</td>
<td></td>
</tr>
<tr>
<td>0 µg/L (control)</td>
<td>9.85 ± 1.99 a</td>
<td>39.9 ± 3.73 a</td>
<td></td>
</tr>
<tr>
<td>75 µg/L</td>
<td>9.30 ± 2.01 a</td>
<td>36.3 ± 2.80 a</td>
<td></td>
</tr>
<tr>
<td>750 µg/L</td>
<td>10.2 ± 3.05 a</td>
<td>34.6 ± 7.67 a</td>
<td></td>
</tr>
</tbody>
</table>

Values in the same column with the same letter are not significantly different among treatments (p > 0.05) according to one way ANOVA test.
There are limited experiments taking the rice grains into the account when studying the uptake of PBDEs by rice plants, which may be due to the hydrophobic property of PBDEs and the reported affinity of PBDEs to root lipids (Huang et al., 2010). Therefore, very few studies reported the accumulation of PBDEs in rice grains, which involves a longer translocation distance. Nevertheless, rice grains can play an essential role in PBDEs exposure in the food chain. In addition, being a storage organ of rice plants, rice grains may also be significant in bioaccumulation of PBDEs which are taken up by other parts of rice. Therefore, the biomass of rice grains and concentration of PBDEs in rice grains can provide a more complete picture in remediation study. To the best of our knowledge, it is the first wastewater treatment study investigated the uptake of PBDEs in rice grains. There was no difference in the rice grains yields between the control and wastewater treatment wetlands. The yielding rate was 66.7% on average with no significant difference between the controls and treatments (p > 0.05) (Table 5.5).

5.3.4.2 Effects on Treating Artificial BDE-209 Wastewater in the Combined System

After being treated by the TiO₂ photocatalysis, the concentration of BDE-209
was further lowered in the constructed wetlands system (Fig. 5.3). The concentrations of BDE-209 in water samples in the unplanted control were relatively constant when compared with the planted treatments, except that with initial BDE-209 concentration of 750 µg/L increased slightly with time (Fig. 5.3). This might be due to the contamination of water samples by desorption of BDE-209 adhered on the wall of the tank from previous wastewater. Generally, there was around 40% loss of BDE-209 in the water samples in the unplanted treatment, which might probably be due to photolysis by sunlight, sorption to soil and evaporation (Söderström et al., 2004).

Compared to the unplanted controls, the planted treatments with both rice and reed significantly promoted the degradation of BDE-209 in water samples (p < 0.05) (Fig. 5.3). The average removal percentage of BDE-209 in pre-treated wastewater by rice was 82.5 ± 1.08%, and reed was 85.5 ± 2.97%. The concentrations of BDE-209 in water samples in the treatment tank planted with reed declined steadily until the end of the experiment, which were different from those in rice treatments (Fig. 5.3). There were slight increases in the curves of water samples in rice treatments at the end, indicating a slight increase of BDE-209 in the wastewater. Since rice had already reached senescence at 120-day, the ability of treating BDE-209, particularly under high level (~354 µg/L) might
Figure 5.3 Concentrations of BDE-209 in water samples against time. Values with asterisk (*) on the unplanted control curves are significantly different from the planted treatments according to independent T-test (p < 0.05).
thus decline. It was also observed that a small peak of BDE-209 content was found at 90-day during flowering, but this increase was not significant (p > 0.05) (Fig. 5.3).

The PBDE congeners profiles of the photocatalytically and combined system treated wastewater are illustrated in Fig. 5.4. It was found that the portions of BDE-209 in the water samples from the planted combined systems were lower than those in the photocatalytically treated and unplanted samples (Fig. 5.4). Meanwhile, the proportions of the lower brominated PBDEs (mono- through nona-BDEs) increased in the planted treatments when compared with the non-planted ones and the photocatalytically pre-treated influent. It was probably due to the degradation of BDE-209 by sunlight and plants. A similar phenomenon was observed in the unplanted controls when compared to the influent of the combined system (photocatalytically pre-treated PBDEs wastewater). The percentages of mono-, di- and nona-BDEs became higher, which might be a result of photolysis of BDE-209 and other lower molecular PBDEs (Söderström et al., 2004). The percentages of BDE-209 in the profile of wastewaters with initial BDE-209 concentration of 750 µg/L were generally higher than those of 75 µg/L in all treatments (Fig. 5.4), which might indicate a slower debromination rate of BDE-209 in wastewater with higher initial BDE-209 content (750 µg/L).
Figure 5.4 Degradation profile of BDE-209 in water samples collected from the combined system after 6-d retention period. The values in parentheses are the initial concentrations of BDE-209 in µg/L.
5.3.4.3 Fate of BDE-209 in the Combined System

The residual concentrations of BDE-209 in soil and plant tissues (root, shoot and rice grains) after harvest are illustrated in Tables 5.7 and 5.8. The pre-treated BDE-209 wastewater was discharged into the constructed wetland and BDE-209 deposited in the soil. The BDE-209 content in soil increased with the initial concentrations in the wastewater (Table 5.7). Compared to the unplanted control, the BDE-209 concentrations in soil planted with rice and reed plants were significantly lower in both 75 and 750 µg/L of initial BDE-209 concentrations (p < 0.05) (Table 5.7). It was evident that planting with rice and reed plants significantly enhanced the dissipation of BDE-209 in soil received pre-treated BDE-209 wastewater.

Different plant organs (root, shoot and rice grain, rice hull) were tested for PBDE levels and it was observed that BDE-209 was accumulated in both rice and reed plants (Table 5.8). There were generally higher BDE-209 concentrations detected in roots than shoots of both rice and reed plants (Table 5.8). This could be explained by the highly hydrophobic property of BDE-209 (log $K_{ow} = 9.97$), and thus BDE-209 was more likely to be retained in the root rather than translocated to the shoot through transpiration (Huang et al., 2010). There was no significant difference in the BDE-209 concentrations in plant tissues (root and
Table 5.7 Concentrations of BDE-209 in soil planted with rice and reed plants associated with AMF in the combined system

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Initial BDE-209 (75 µg/L)</th>
<th>Initial BDE-209 (750 µg/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Unplanted</td>
<td>19.4 ± 3.70 a</td>
<td>171 ± 12.4 a</td>
</tr>
<tr>
<td>Rice</td>
<td>9.21 ± 2.84 b</td>
<td>88.0 ± 24.6 b</td>
</tr>
<tr>
<td>Reed</td>
<td>7.25 ± 3.60 b</td>
<td>90.7 ± 15.7 b</td>
</tr>
</tbody>
</table>

Values in the same column with the same small letter are not significantly different in the same column (p > 0.05) according to one way ANOVA test.
Table 5.8 Accumulation of BDE-209 in plant tissues of rice and reed plants

<table>
<thead>
<tr>
<th>Plants</th>
<th>Treatment</th>
<th>Initial BDE-209 concentration (µg/L)</th>
<th>Root</th>
<th>Shoot</th>
<th>Grains</th>
<th>Hulls</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rice</td>
<td>75</td>
<td>105 ± 28.2 c</td>
<td>4.56 ± 0.925 c</td>
<td>13.8 ± 1.70</td>
<td>28.4 ± 2.48*</td>
<td></td>
</tr>
<tr>
<td>Reed</td>
<td>75</td>
<td>96.1 ± 11.0 c</td>
<td>3.24 ± 0.649 c</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Rice</td>
<td>750</td>
<td>728 ± 39.6 a</td>
<td>81.7 ± 9.72 a</td>
<td>41.0 ± 8.06</td>
<td>74.7 ± 3.59*</td>
<td></td>
</tr>
<tr>
<td>Reed</td>
<td>750</td>
<td>552 ± 99.0 b</td>
<td>40.5 ± 10.5 b</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Values in the same column with the same small letter are not significantly different ($p > 0.05$) according to one way ANOVA test. Values with asterisk (*) are significantly different from the BDE-209 concentrations in rice grains according to independent T-test ($p < 0.05$).
shoot) of rice and reed plants (p > 0.05) when the initial concentration of BDE-209 wastewater was 75 µg/L (Table 5.8). However, when the concentration increased to 750 µg/L, the variation between rice and reed became significant (p < 0.05), and the concentration of BDE-209 in rice (728 nmol/kg) was higher than those in reed (552 nmol/kg) (Table 5.8). The total concentrations of BDE-209 in the two plants (on whole plant basis) are presented in Table 5.9 and no significant difference could be observed between the total BDE-209 in rice and reed plants (p > 0.05). Referring to the biomass data of rice and reed plant listed in Tables 5.5 and 5.6, it was found that the total biomass of reed (root and shoot) was significantly higher than that of rice (p < 0.05). Therefore, the lower BDE-209 content accumulated in reed was probably the result of dilution effect by biomass.

The concentrations of BDE-209 in rice grains and hulls were also detected (Table 5.8). It was found that the concentrations of BDE-209 in rice grains and hulls increased with its initial concentration in wastewater, though it was not proportionally (Table 5.8). The BDE-209 levels in rice grains (75 µg/L = 13.8 nmol/kg) was significantly lower than that in hulls (75 µg/L = 28.4 nmol/kg) (p < 0.05) (Table 5.8). Du et al. (2013), investigated the PBDEs levels in different parts of rice plants (including leaf, stem, shell and grain) cultivated in lysimeters spiked with BDE-209 (23.3 mg/kg), and found that the BDE-209 concentrations in rice
Table 5.9 The total concentrations of BDE-209 in the rice and reed plants (on whole plant basis) under two initial BDE-209 concentrations

<table>
<thead>
<tr>
<th>Plants</th>
<th>Initial BDE-209 (75 µg/L)</th>
<th>Initial BDE-209 (750 µg/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rice</td>
<td>0.966 ± 0.254</td>
<td>7.50 ± 1.65</td>
</tr>
<tr>
<td>Reed</td>
<td>1.02 ± 0.269</td>
<td>6.99 ± 1.86</td>
</tr>
</tbody>
</table>
hulls could be > 3 times higher than that in rice grains. Fu et al. (2012) collected rice grains from Fengjiang town (one of the most intensive e-waste dismantling sites in Taizhou, China) from 2005 to 2009, and suggested that the elevated PBDEs level in rice hulls might be ascribed to the exposure to atmospheric PBDEs.

5.3.4.4 Suggested Physiological Uptake Mechanisms of PBDEs in Rice and Reed Plants

There are two major strategies for phytoremediation of organic contaminants, which are direct uptake and phytoremediation ex planta (secretion of exudates which support the growth and metabolic activities of microbes in the rhizosphere) (Alkorta and Garbisu, 2001). In the phytodegradation of PBDEs by enzymes in root exudates, nitroreductase (NaR) and glutathione-transferase (GST) may be involved in the extracellular degradation and debromination (Huang et al., 2013). The debrominated PBDEs were more readily taken up by plants and translocated to other parts of plants (Huang et al., 2011). The phytodegraded PBDEs were usually taken up by roots through passive transport and/or active transport. In an investigation of the accumulation of PCBs and DDT (which are structurally similar to PBDEs) by common reed and rice plants, it was found that the uptake could be by both passive and active transports with passive adsorption before
active transport (Chu et al., 2006a). Chapters 3 and 4 indicated that passive transport (adsorption and/or diffusion across the roots) was a significant uptake approach in rice uptake of PBDEs. In addition, it was also found that the PBDEs entered the rice roots with the water stream mainly through the apoplastic pathway (Chapters 3 and 4). Wild et al. (2005) observed the entry of anthracene into the epidermal cells (of maize) was radial. The anthracene stream entered and moved through the cell walls of cortex cells towards the shoot by diffusion, but it did not enter the vascular transport system (phloem/xylem) (Wild et al., 2005). A small portion of anthracene then moved laterally through two or three cells across the cell vacuoles and entered the symplastic flow (Wild et al., 2005). Having similar physiochemical properties to PAHs, the uptake of PBDEs in plants would probably be similar to those of PAHs, which were mainly through the apoplastic pathway in the cortex.

5.3.4.5 PBDE Congeners Profile in Soil and Plants

Lower-brominated PBDE congeners were detected in all soil and plant tissue samples, indicated debromination of PBDEs and/or uptake of lower molecular PBDEs by the soil-plant system occurred (Figures 5.5 and 5.6). The PBDE congeners profile of unplanted soil was similar to that of the pre-treated waste water with a high portion of BDE-209 (> 40%), which might be due to the high
Figure 5.5 Degradation profile of BDE-209 in soil planted with rice and reed. Unplanted refers to unplanted control soil fed up with BDE-209 wastewater. Rice refers to soil planted with rice plant (Hefengzhan), and reed refers to soil planted with reed plant. The values in parenthese are the initial concentrations of BDE-209 in µg/L.
Figure 5.6 Degradation profile of BDE-209 in rice and reed. All the congener profiles were in the treatments fed up with BDE-209 wastewater of initial concentration at 75 µg/L.
log $K_{ow}$ value of BDE-209 (9.97) (Fig. 5.4). The highly hydrophobic BDE-209 has a strong sorption to soil, especially soil organic matter, so it tends to deposit in soil (Huang et al., 2011). The proportion of BDE-209 decreased when the system was planted with rice and reed plants, while the percentage of the lower brominated congeners (mono-BDE through octa-BDE) increased (Fig. 5.5).

There was no significant difference in the PBDE congeners profiles of soil grown with rice and reed plants ($p > 0.05$). The percentage of BDE-209 increased drastically when the BDE-209 concentration in wastewater increased (Initial concentration = 750 $\mu$g/L) (Fig. 5.5). The increase of BDE-209 portion might be due to the decreased degradation rate of BDE-209 in soil when the concentration in wastewater increased, while some of the lower molecular weight PBDEs were taken up by the plants. However, the BDE-209 (~354 nmol/L) was constantly fed into the constructed wetland and accumulated in the soil. The plant might not be capable to deal with the regular influx of BDE-209 in a short time (6 days). This suggests that the debromination pattern was affected by the concentration of BDE-209 in the influents.

The PBDE congeners profiles of plant tissues are presented in Fig. 5.6. There was no significant difference between the PBDE profiles of 75 and 750 $\mu$g/L treatments, and therefore only the plant tissues involved in 75 $\mu$g/L treatments are
included in Fig. 5.6. Generally, the PBDEs uptake patterns in rice and reed were similar. Mono- and deca-BDE occupied the majority of the congeners profile of rice and reed roots (Fig. 5.6). Compared to the PBDEs profile of rice (Hefengzhan) roots grown in BDE-209 spiked sand in Chapter 3, the pattern was similar (with BDE-209 be one of the dominant congeners), except mono-BDE was in a higher portion in the constructed wetland system than in the spiked sand. This was probably because the BDE-209 wastewater was pre-treated by an aqueous TiO$_2$ suspension in visible light, with BDE-209 degraded into lower brominated congeners (mainly tetra- and penta-BDEs). These congeners were more bioavailable for plant uptake and could be further debrominated into mono- and di-BDEs in roots (Huang et al., 2011). The number of congeners increased when the PBDEs were translocated from roots to shoots (Fig. 5.6). The percentage of deca-BDE apparently decreased (~4.5%) and mono-BDE increased (~75%) (Fig. 5.6). This change was probably due to the metabolism and further debromination of BDE-209 and other weathered PBDEs from roots and shoots.

Although the portion of BDE-209 in rice grains (15.6%) was higher than in rice hulls (4.07%), the total PBDEs (grains = 88.3 nmol/kg; hulls = 698 nmol/kg) and BDE-209 content (grains = 13.8 nmol/kg; hulls = 28.4 nmol/kg) of rice hulls were significantly higher than those of rice grains (p < 0.05). This phenomenon
could be explained by the extensive surface area and lipid content of rice hulls. Fu et al. (2012) stated that rice hulls were highly porous with the outer surface area about 4000 m²/m³. Tao et al. (2006) also mentioned that the lipid content of rice hulls could be as high as 10.1 mg/g d.w. Therefore, these provide rice hulls with a higher capacity for adsorption of hydrophobic organic contaminants, including PBDEs, than rice grains.

5.3.4.6 Comparison of the BDE-209 Removal Efficiencies of TiO₂ Photocatalysis, Phytoremediation and the Combined System

The final reduced percentage of BDE-209 in the wastewater in the combined system was calculated and compared with those in TiO₂ photocatalysis and phytoremediation. The removal rate of BDE-209 in TiO₂ (mixture of anatase (65%) and rutile (35%) crystalline forms) aqueous suspension under visible light (with pH 7, humic acids at 5 mg/L) for three days was 55.5 ± 7.45%. Removal of BDE-209 in sand by plants exhibited a percentage loss of BDE-209 in sand planted with rice (cultivar: Hefengzhan; AMF: GI, pH: 7) of 63.4 ± 0.326%, while that in soil planted with reed (Reed; AMF: GI, pH: 7) 48.1 ± 10.4%. In the combined system, there was no significant difference of BDE-209 reduction rates between the two treatments with initial two different BDE-209 concentrations (75 and 750 µg/L) (p > 0.05). The average overall removal percentage of BDE-209 in
the wastewater was 93.6 ± 2.19% (75 µg/L) and 92.1 ± 1.11% (750 µg/L) in the
combined system, which was apparently higher than the individual TiO$_2$
photocatalysis and phytoremediation treatments.

5.4 Conclusion

The present study has highlighted the feasibility of treating BDE-209
contaminated wastewater with the combined photocatalysis/wetland system.
Similar to rice (Oryza sativa), reed (Phragmites australis) could also accumulate
and degrade BDE-209 within the plants. Although the BDE-209 levels in rice
grains (the most common consumed part of rice) were significantly lower than in
hulls, the considerable amount of PBDEs in rice grains might be a concern in the
use of rice plants in the wastewater treatment. The combined system could
eventually increase the removal percentage of BDE-209 in wastewater and lower
the proportion of higher brominated congeners. These findings agree with the
hypothesis that the combined system is more efficient in the removal of BDE-209
than the individual systems, which can be explained by the enhanced
bioavailability of PBDEs for plant uptake.
CHAPTER 6
GENERAL DISCUSSION AND CONCLUSIONS

6.1 Introduction

Polybrominated diphenyl ethers (PBDEs) are brominated flame retardant (BFR) chemicals extensively used in electronic equipment such as computers and television sets (WHO, 1994). These brominated flame retardant products include commercial pentabromodiphenyl ethers (penta-BDEs and tetra-BDEs), commercial octabromodiphenyl ethers (hexa-BDEs, hepta-BDEs and octa-BDEs) and decabromodiphenyl ether (deca-BDE) (Rahman et al., 2001). Due to the increasing health concerns to humans and the environment about application of PBDEs as flame retardants, the EU has banned the uses of penta-BDEs and octa-BDEs in all applications for EU market since 2004 (BSEF, 2013). China has also started to restrict the use of deca-BDE in manufacture of phones and printers but not in other electrical and electronic appliances (BSEF, 2013).

With persistent and semi-volatile properties, PBDEs can travel long distances in air and water (de Wit, 2002). They can be bioaccumulated in seafood and animal tissues and subsequently biomagnified in food chains (de Wit, 2002). Attention should be especially given to BDE-209 which is the major component
of the commercial deca-BDE flame retardant (97–98%) (Wang et al., 2007). Among 209 congeners, deca-BDEs contribute to the majority of production and consumption (30000 tonnes/year), which is about 75% (Sjödin et al., 1999). Generally, BDE-209 is believed to be less toxic to rats than the other lower brominated congeners in terms of NOEL (no-observed-effect-level) values. In rodent studies of subchronic toxicity, NOEL values are usually in < 10 mg/kg/day for pentaBDE, but much higher for decaBDE (g/kg/day range) (Costa and Giordano, 2007). Nevertheless, degradation of BDE-209 probably leads to prominent production of BDE-47 and 99, which are two PBDE congeners that attract the most public concern due to their toxicity (thyroid hormone disruption and reproductive toxicity in rodent tests) and persistence (Sun et. al., 2009; Zhou et al., 2002; Kuriyama et al., 2005).

TiO$_2$ photocatalysis and phytoremediation are two promising technologies to treat organic pollutants, including PBDEs in soil and aqueous solutions (Huang et al., 2013; Huang et al., 2011). Although there has been limited study about TiO$_2$ photocatalysis of PBDEs under visible light, degradation of halogenated organic contaminants, such as $p,p'$-DDT, could be achieved by photocatalysis with humic substances (HS)-sensitized TiO$_2$ under visible light (Zhao et al., 2004). Italian ryegrass (*Lolium multiflorum* L.) inoculated with an AM fungus (*Glomus mosseae*)
contributed to BDE-209 loss in soil up to 56.4% (Wang et al., 2011). Nevertheless, these two technologies have their own limitations and drawbacks (Comninellis et al., 2008; Cunningham, 1995), and therefore a combined approach involving these two technologies was proposed.

In the combined photocatalysis and constructed wetland (phytoremediation) system, both technologies can be complement of each other, leading to synergistic effects in treating organic contaminants in wastewater in several studies. Herrera-Melián et al. (2012) reported the degradation of 4-nitrophenol (4NP) in the combined system, and observed 100% removal of 4NP and 49.5% removal of TOC using the combination of solar TiO$_2$-photocatalysis and CWs (Phragmites sp., Cyperus sp., Pontederia sp., and Scirpus sp.) (Herrera-Melián et al., 2012).

In the present study, TiO$_2$-photocatalysis, phytoremediation and the combined system of these two technologies were employed for treating PBDEs contaminations. The major objectives of the current study were to (1) investigate the abilities of TiO$_2$ photocatalysis (under visible light) and phytoremediation (Oryza sativa (rice) and Phragmites australis (common reed)) to degrade BDE-209; (2) evaluate the root uptake mechanisms of PBDEs (BDE-47, -99 and -209) by rice cultivars; (3) examine the removal efficiency of BDE-209 in the combined TiO$_2$ photocatalysis/constructed wetland system.
6.2 PBDEs in Hong Kong Sewage Treatment Works

Due to the rapid global industrial and economic development, the consumption and production of PBDEs have been growing drastically. The domestic production of BFRs in China was 10,000 metric-tonnes in 2000, of which commercial deca-BDE was one of the dominant products (Jin et al., 2009). Uncontrolled recycling of electronic-waste (e-waste) has also exerted human and environmental health risks to PBDEs exposure in China. Located at the mouth of the Pearl River estuary, Hong Kong is subjected to the potential risks through the discharged wastewater containing PBDEs from the Pearl River Delta (PRD), in addition to the local illegal e-waste recycling problem (Guan et al., 2007; Man et al., 2011). Although the Basel Ban was incorporated into the Waste Disposal Ordinance in April 2006, transit of e-waste and recycling activities are not totally banned if an import license is obtained in Hong Kong (HK EPD, 2010; Wang et al., 2013). E-waste recycling and open burning activities contribute to significant sources of PBDEs in soil (Man et al., 2011), which may subsequently enter the sewage drainage system through surface-water runoff by rain.

Most of the existing conventional wastewater treatment facilities are not well designed for treating emerging contaminants (including PBDEs) in municipal wastewater, and therefore these chemicals may eventually enter water bodies and
even food chains (Bolog et al., 2009). It has been estimated that PBDEs have been discharged at 2280 kg/year (predominated by BDE-209) through wastewater discharged from the Pearl River Delta (Peng et al., 2009). A study about the release of PBDEs in the sewage treatment works in Hong Kong has been conducted recently and the PBDE congeners profile of the sewage collected from two sewage treatment works. These profiles from Stonecutters Island Sewage Treatment Work (SCISTW) (which adopts a chemically enhanced primary treatment (CEPT) process) and Shatin Sewage Treatment Work (STSTW) (with a biological activated sludge treatment process) are illustrated in Fig. 6.1. BDE-209 was one of the dominant congeners through the treatment processes (including influent, sludge and effluent) at both SCISTW and STSTW (Table 1.3 and Fig. 6.1) (Man et al., 2013). Although the removal rate of BDE-209 in influent was high (SCISTW = 96.0 ± 2.62%; STSTW = 96.2 ± 2.41%), the majority of BDE-209 was deposited in sludge due to its strong sorption to solids (Table 1.3) (Man et al., 2013; Peng et al., 2009). There were also about 3.63 and 0.267 kg per year of PBDEs (Σ14) released from SCISTW and STSTW respectively through discharging of effluent (Man et al., 2013). High portions of BDE-47 and BDE-99 in the effluents of both STWs noted were probably derived from debromination of BDE-209 in the sewage treatment process. This would be another concern in
Figure 6.1 PBDE congeners profile in sewage samples from two STWs in Hong Kong. CS-T refers to crude sewage (with particulate matter); PE-T refers to primary effluent (with particulate matter) after primary treatment; FE-T refers to final effluent (with particulate matter). SCI refers to sewage from Stonecutters Island Sewage Treatment Work (SCISTW). ST refers to sewage from Shatin Sewage Treatment Work (STSTW) (Man et al., 2013).
marine contamination receiving sewage effluents. For these reasons, this study aimed at exploring an advanced wastewater treatment technology (combined photocatalysis and constructed wetland systems), which might help to degrade and eliminate BDE-209 in the wastewater, and thus reduce the risks of marine contamination by discharging these incompletely or partially treated wastewater containing PBDEs.

6.3 TiO$_2$ Photocatalysis of BDE-209

6.3.1 ROS Production and Half-Lives of Degradation of BDE-209 in Aqueous TiO$_2$ Suspension under Visible Light

It has been noted that the TiO$_2$-assisted degradation of the organic laser dye (squarylium cyanine dye (SQ)) in aqueous TiO$_2$ suspensions (4 mg/50 mL) under visible light could be achieved by •OH radical production, and then forming indolenium-2-one species (Wu et al., 1999). Therefore, the hydroxyl radical production in the TiO$_2$ and BDE-209 mixtures under different conditions were measured by dichlorofluorescein (DCF) assay by using dichlorofluorescein diacetate (H$_2$DCF-DA) in the present study (Chapter 2). The hydroxyl radical level remained at least twice that of the control level during the 6-day incubation. In addition, the residual PBDE congeners after photocatalytical degradation of BDE-209 by TiO$_2$ were identified, with tetra- and penta-BDEs the dominant
degraded products of BDE-209. The photocatalytical degradation of BDE-209 followed the first-order rate kinetics in the first 6 days, with a half-life of 3.05 days (Chapter 2).

6.3.2 Identification of Optimal Operation Conditions for TiO₂ Photocatalytic Degradation of BDE-209 under Visible Light

Optimum conditions for photocatalytical degradation of BDE-209 were found at pH 12 (93 ± 1%), 5-20 mg/L (93.0 ± 1.70%, 91.6 ± 3.21%, 91.9 ± 0.952%, respectively), of humic acids (HA) and in the form of anatase/rutile TiO₂ (82 ± 3%). It could be concluded that the photocatalytical degradation of BDE-209 was the most efficient at pH 12, with humic acid concentration at least of 5 mg/L, and in the mixed crystalline form of TiO₂ (anatase (65%) and rutile (35%)).

Degradation of BDE-209 can be attributed to the production of hydroxyl radicals (Peterson et al., 1991), which are more readily generated under alkaline conditions (higher pH, e.g. 12) (Zheng et al., 1997). In this experiment, humic acid was added as photosensitizer in order to extend the absorption spectra into the visible light region (Vinodgopal, 1994). The HA molecules were excited by absorbing visible light and donated electrons to the conduction band (CB) of TiO₂ (Cho and Choi, 2002). The sensitized reduction process can be subsequently
propagated through the oxidation of HA and release of electrons to the substrates (e.g. PBDEs) (Cho and Choi, 2002). The natural crystalline forms of TiO$_2$ included anatase, rutile and mixture of anatase and rutile (Tsuji et al., 2006). In the mixture of anatase and rutile forms of TiO$_2$, anatase might act as an oxidation centre, whilst rutile might act as a reduction centre, where rutile could accept electrons from anatase (Hongo and Nogami, 2007). The electrons accumulated in rutile could then be transferred to reduction reactions for production of a series of reactive oxygen species (ROS) and continue the photocatalytic process (Hongo and Nogami, 2007).

6.4 Plant Uptake and Dissipation of PBDEs

6.4.1 Selection of PBDE Congeners

According to the results of Chapter 2, tetra- and penta-BDEs were the two dominant PBDE groups in the products of photocatalytic degradation of BDE-209 in aqueous suspension of TiO$_2$. Therefore, BDE-47 and -99, the two representative congeners of tetra- and penta-BDEs frequently detected in the human body (US EPA, 2008a, b), were chosen, in addition to BDE-209, for the plant uptake study.

6.4.2 Uptake, Translocation and Dissipation of PBDEs in Rice (Oryza sativa) Associated with Arbuscular Mycorrhizal Fungi (AMF)

The fate and uptake of BDE-209 were investigated in three common lowland
rice cultivars (Fengmeizhan, Hefengzhan and Guangyinzhan) associated with three different species of AMF (*Glomus intraradices*, *Glomus mosseae* and *Glomus versiforme*) in Chapter 3. In general, there was no significant difference in the mycorrhizal infection rates between the three rice cultivars \( (p > 0.05) \) (Table 3.1). Spiking of BDE-209 did not exert any significant effect on the mycorrhizal infection rates in rice roots \( (p > 0.05) \) (Table 3.1). The three tested AMF species, in descending order of mycorrhizal root colonization rates were GI > GM > GV (Table 3.1). The lowest colonization rate was found at pH 8 in some treatments (e.g. Hefengzhan GI (ctrl) at pH 8 = 13.8%) with GI (Table 3.1). This might be because prevalent AMF species often vary with the ambient environment, including pH, for optimal germination and growth (Smith and Read, 2008). Van Aarle et al. (2002) elucidated this with a relatively lower arbuscule and vesicle formation in *G. intraradices* at pH 6 than pH 5. Root colonization by AMF significantly promoted the root and shoot biomass of most of the treatments \( (p < 0.05) \) (Tables 3.2 and 3.3). The root colonization rate was also significantly correlated with both the root lipid and soil DOC contents of Hefengzhan treatment \( (r = 0.622, p < 0.01 \text{ and } r = 0.602, p < 0.01, \text{ respectively}) \). These suggested that the AMF colonization rate had a close relationship with the biomass, the root lipid content of rice plants and DOC content of sand.
Secilia and Bagyaraj (1992) attributed the abilities of AMF (include *Glomus intraradices, Glomus mosseae and Glomus versiforme*) to enhance the biomass of rice (Prakash) in a soil with low level of phosphate (25 kg/ha) to the increased bioavailability of some nutrients, in particular inorganic (ortho)phosphate (Smith and Read, 2008). The uptake of phosphate was related to the expression of the rice phosphate transporter gene, OsPT11, during the rice root-mycorrhizal symbiosis (Paszkowski et al., 2002). In addition, the absorption of nutrients, especially phosphate, could be promoted by the extraradical mycelia of AMF (Smith et al., 2001). These suggested that AMF plays a significant role in acquiring phosphate for the host plant during the mycorrhizal symbiosis.

A significant relationship was found between the mycorrhizal colonization rates and the root lipid contents (Hefengzhan: $r = 0.622, p < 0.01$) in Chapter 3. It was reported that the concentrations of neutral lipids (include sterol esters, triglyceride, free fatty acids, diglyceride and sterols) were higher in mycorrhizal infected roots than the uninfected roots in clover (Cooper and Lösel, 1978). Furthermore, the transcript level of the lipid transfer protein (LTP) gene was also increased by the root colonization of *Glomus mosseae* (Blilou et al., 2000). In addition, Bago et al. (2003) noted that AMF obtained photosynthetically fixed carbon mainly in the form of lipid (triacylglyceride) and carbohydrate (glycogen)
from their host plants. These might explain the close relationship between the accumulated lipids in mycorrhizal roots and the rice root-fungi symbiosis.

The significant correlation between AMF colonization rates and DOC content in sand described in Chapter 3 (r = 0.602, p < 0.01) might be related to the secretion of root exudates (Bécard and Piché, 1989). A close relationship was also reported between percentage root length infected after 36-day incubation and soluble carbohydrate concentrations within roots after 24-day incubation (Same et al., 1983). These soluble carbohydrates could be released through root cell membrane and the net loss of these substances from the roots (Citrus aurantium L.) enhanced the growth of mycorrhizal fungus (Glomus fasciculatus), so allowing the AMF to improve root phosphorus nutrition (Graham et al., 1981).

Planting with rice plants (non-mycorrhizal or inoculated with AMF) significantly lowered the residual BDE-209 content in sand, especially in the Hefengzhan (pH 7) treatment, than the unplanted treatments (p < 0.05) (Table 3.6). The highest concentration of BDE-209 was also always accumulated in plant tissues (roots and shoots) of Hefengzhan at pH 7 (Fig. 3.4). A positive significant correlation was found between BDE-209 concentration in roots and root lipid contents of AMF inoculated rice roots (r = 0.425, p < 0.05). There were also generally higher BDE-209 concentrations in rice roots than shoots (p < 0.05),
indicating that hydrophobic BDE-209 ($K_{ow} = 9.97$) tended to be taken up and accumulated in rice roots than shoots. A significant correlation was observed between the concentrations of BDE-209 in plant tissues (roots and shoots) of Hefengzhan and the root colonization rates ($r = 0.731, p < 0.01$). It was probably because the hyphae of AMF (e.g. *Glomus mosseae* and *Glomus etunicatum*) can absorb organic contaminants (e.g. PAHs and BDE-209) and transport them to roots of the host plant (e.g. ryegrass), enhancing the accumulation and uptake of the organic contaminants (Gao et al., 2010; Wang et al., 2011).

Based upon the results from Chapter 3, uptake and dissipation of BDE-47 and -99 were investigated in Hefengzhan associated with AMF (GI, GM and GV) at three pHs (pH 6, 7 and 8). The interactions between AMF colonization and plant growth in BDE-47 and -99-contaminated sand were generally the same to those reported in Chapter 3 (Chapter 4). Table 6.1 compares PBDEs removal percentages in the growth substrate (sand/soil) of rice and reed plants. It was shown that not all AMF inoculated treatments could lead to enhanced removal of PBDEs removal when compared with the NAMF control. Generally, the rice plant associated with GI demonstrated the highest removal percentages of BDE-47, -99 and -209 between the three AMF ($p < 0.05$). The three PBDE congeners in ascending order of removal percentages in sand are: BDE-47 < BDE-99 <
BDE-209 (Table 6.1). This was possibly due to the extent of congener specific sorption in the growth substrates and/or degradability and uptake by plants (Mueller et al., 2006), and the molecular size and $K_{ow}$ values of the congeners (Cunningham and Ow, 1996).

Soil organic matter (SOM) content plays a significant role in the bioavailability of BDE-209 and thus affects its removal from soil (Cunningham and Ow, 1996). A study indicated that the SOM content of soil can affect the accumulation of dieldrin by carrots grown in it (Harris and Sans, 1967). Therefore, the performance of rice and reed plants in BDE-209 reduction in the two types of growth substrates (sand and soil) would be more accurately compared by the root concentration factor (RCF) of rice (Chiou et al., 2001). This was defined as the ratio of the PBDEs concentrations in roots and in soils, and was obtained for BDE-47, -99 and -209 in rice (sand, Hefengzhan, pH 7) and reed plants (soil, pH 7) (Table 6.2) (Gao and Zhu, 2004). A decreasing trend was observed in RCFs from BDE-47 to BDE-209, which could be explained by the increasing log $K_{ow}$ values of PBDE congeners (BDE-47: 6.81; BDE-99: 7.32; BDE-209: 9.97) (Table 6.2) (ATSDR, 2004b; Gao and Ling, 2006). Physiochemical properties of PBDEs, such as log $K_{ow}$, might affect their mobility in soil organic matter and sorption to root surface which is an essential route for root uptake of hydrophobic organic
Table 6.1 Removal percentages of BDE-47, -99 and -209 in sand/soil grown with rice (Hefengzhan) or reed at pH 7

<table>
<thead>
<tr>
<th>Treatments</th>
<th>BDE-47</th>
<th>BDE-99</th>
<th>BDE-209</th>
<th>BDE-209</th>
</tr>
</thead>
<tbody>
<tr>
<td>NAMF</td>
<td>40.0 ± 3.91 Bb</td>
<td>48.1 ± 0.260 ABc</td>
<td>52.6 ± 1.06 Ab</td>
<td>46.1 ± 11.1 AB</td>
</tr>
<tr>
<td>GI</td>
<td>53.1 ± 1.73 BCa</td>
<td>57.8 ± 2.32 ABa</td>
<td>63.4 ± 0.199 Aa</td>
<td>49.8 ± 6.38 C</td>
</tr>
<tr>
<td>GM</td>
<td>43.3 ± 5.48 Bb</td>
<td>52.2 ± 0.216 Ab</td>
<td>52.1 ± 3.15 Ab</td>
<td></td>
</tr>
<tr>
<td>GV</td>
<td>46.5 ± 5.38 Bab</td>
<td>47.2 ± 2.55 Bc</td>
<td>55.5 ± 3.26 Ab</td>
<td></td>
</tr>
</tbody>
</table>

NAMF refers to the non-mycorrhizal control; GI refers to *Glomus intraradices*; GM refers to *Glomus mosseae*; GV refers to *Glomus versiforme*. Values with the same small letter are not significantly different among different AMF species in the same column. Values with the same capital letter are not significantly different in the same row under one-way ANOVA test (p > 0.05).
contaminants (Cunningham and Ow, 1996; Gao and Ling, 2006).

6.4.3 Uptake Mechanism of PBDEs in Rice Plants

A partition-limited model was used for estimating and describing the approach of the uptake of BDE-209, -47 and -99 by rice roots (Chapters 3 and 4) (Chiou et al., 2001). In the partition-limited model, the tendency of a particular contaminant to reach equilibrium between plant (or a part of it) and growth substrates (sand) at a specific time can be described by quasi-equilibrium factor \( \alpha_{pt} \) (Chiou et al., 2001). The model can be equated to a function of the contaminant partition coefficients \( K_{ow} \) and \( K_{som} \), the compositions of plant (water \( f_{pw} \) and organic matter \( f_{pom} \)), the concentration of a contaminant in specific part of plant (or the whole plant) \( C_{pt} \) and the contaminant concentration in sand \( C_s \) (Chapter 3) (Chiou et al., 2001). The results of the uptake of BDE-47, -99 and -209 by rice roots estimated by the partition-limited model are shown in Table 6.3 (Chiou et al., 2001).

The \( \alpha_{pt} \) values of the PBDEs studied were all < 1, indicating that a passive transport dominated uptake (Chiou et al., 2001). Highly hydrophobic organic contaminants, including PBDEs, tend to adsorb onto SOM, and therefore the bioavailability of PBDEs in sand in the present study was related to their affinity to SOM (Gao and Ling, 2006). A significantly lower \( \alpha_{pt} \) value of BDE-209 was
Table 6.2 Root concentration factors (RCFs) \( (C_r/C_s) \) of BDE-47, -99 and -209 in rice (Hefengzhan, pH 7) and reed on dry weight basis.

<table>
<thead>
<tr>
<th>Treatments</th>
<th>BDE-47</th>
<th>BDE-99</th>
<th>BDE-209</th>
<th>BDE-209</th>
</tr>
</thead>
<tbody>
<tr>
<td>NAMF</td>
<td>1.07 ± 0.336 Ab</td>
<td>0.964 ± 0.140 Ab</td>
<td>0.529 ± 0.0127 Bc</td>
<td>0.319 ± 0.0818 B</td>
</tr>
<tr>
<td>GI</td>
<td>2.49 ± 0.230 Aa</td>
<td>1.99 ± 0.115 Ba</td>
<td>1.03 ± 0.0631 Ca</td>
<td>0.376 ± 0.0643 D</td>
</tr>
<tr>
<td>GM</td>
<td>2.21 ± 0.473 Aa</td>
<td>1.77 ± 0.110 Aa</td>
<td>0.706 ± 0.0700 Bb</td>
<td></td>
</tr>
<tr>
<td>GV</td>
<td>1.40 ± 0.509 Ab</td>
<td>1.11 ± 0.125 ABb</td>
<td>0.711 ± 0.0295 Bb</td>
<td></td>
</tr>
</tbody>
</table>

NAMF refers to the non-mycorrhizal control; GI refers to *Glomus intraradices*; GM refers to *Glomus mosseae*; GV refers to *Glomus versiforme*. Values with the same small letter are not significantly different among different AMF species in the same column. Values with the same capital letter are not significantly different in the same row under one-way ANOVA test \( (p > 0.05) \).
also observed than the two other congeners in sand, implying that the movement of BDE-47 and -99 were more likely to reach equilibrium than BDE-209 in sand (p < 0.05) (Table 6.3). The $a_{pt}$ values were in descending order of BDE-47 > BDE-99 > BDE-209, which was in the same order as their $K_{ow}$ values.

In the analysis of the movement of PBDEs in water into the rice root cells, the uptake coefficients (the ratio of PBDEs concentrations in roots to those in external solutions) of BDE-209, -47 and -99 in dead roots, were higher than those in fresh roots (Chapters 3 and 4). It was evident that the uptake pathways of these contaminants in soil water into root cells were similar and all were apoplastic. When active transport was prevented by heating the rice roots, the uptake process of PBDEs was predominantly by partitioning into lipophilic components of rice roots, such as the root cell wall (Chen et al., 2009). It was reported that phenanthrene possessed a higher sorption capacity in root cells wall rather than in bulk roots due to its lower polarity (Chen et al., 2009). Therefore, PBDEs were more likely retained in root cell walls rather than moving into cytoplasm with the symplastic water stream, due to their high lipophilicity.

6.5 Combined Photocatalysis/Wetland System

6.5.1 Abilities of Rice and Reed in Removal and Accumulation of PBDEs in the Combined System
Table 6.3 The log octanol-water partition coefficients ($K_{ow}$) and quasi-equilibrium factors ($\alpha_{pt}$) of BDE-47, -99 and -209 in the uptake by rice (Hefengzhan) roots.

<table>
<thead>
<tr>
<th>PBDEs</th>
<th>log $K_{ow}$</th>
<th>$\alpha_{pt}$ (sand)</th>
</tr>
</thead>
<tbody>
<tr>
<td>BDE-47</td>
<td>6.81</td>
<td>$1.44 \times 10^{-3} \pm 4.51 \times 10^{-4}$ a</td>
</tr>
<tr>
<td>BDE-99</td>
<td>7.32</td>
<td>$0.966 \times 10^{-3} \pm 1.41 \times 10^{-4}$ b</td>
</tr>
<tr>
<td>BDE-209</td>
<td>9.97</td>
<td>$0.115 \times 10^{-3} \pm 2.78 \times 10^{-6}$ c</td>
</tr>
</tbody>
</table>

Values in the same column with the same small letter are not significantly different ($p > 0.05$) under one-way ANOVA test.
After treating in the HA/TiO2/visible light photocatalysis, over 50% of BDE-209 in the wastewater was reduced in both wastewaters with initial BDE-209 concentrations of 75 and 750 µg/L (Fig. 6.2). The reduction of BDE-209 in this phase of treatment involved both photolysis and TiO₂ photocatalysis with HA sensitization (Cho and Choi, 2002). After that, the effluent was discharged into the constructed wetlands planted with rice and reed plants, respectively (Fig. 6.2). Compared with the unplanted controls, both rice and reed planted treatments could significantly enhance the degradation of BDE-209 in water samples (p < 0.05) (Table 5.4). The concentration curves of reed plant treatments were relatively steady and declined eventually when compared with those of rice plant (Fig. 5.3). There was no significant difference between the removal efficiencies of BDE-209 in the wastewater in rice and reed treatments (p > 0.05) (Table 6.4). The BDE-209 left in the soil after the rice and reed treatments were also not significantly different (p > 0.05) (Table 5.7). A similar observation was made in BDE-209 accumulation in roots and shoots of rice and reed in the wastewater with initial BDE-209 concentration at 75 µg/L (p > 0.05) (Table 5.8). Nevertheless, the amount of BDE-209 accumulated in rice became significantly higher than reed when the initial concentration increased to 750 µg/L (p < 0.05) (Table 5.8), which might be attributed to the dilution effect by the significantly higher biomass of
Figure 6.2 Conceptual flowchart of the combined system with removal rates of BDE-209 in the wastewater. Percentages in bold are the removal rates of BDE-209 in particular individual treatment, while percentages in parenthesis are the removal rates of the whole combined system with specific plant (rice or reed) of different initial concentrations of BDE-209 (75 or 750 µg/L).
reed than rice (p < 0.05).

6.5.2 Comparison of the Individual Systems and the Combined System

The removal percentages of BDE-209 in TiO$_2$ photocatalysis, phytoremediation by rice and reed plants and the combined system were compared (Table 6.5). The removal percentages of BDE-209 in the combined system were found to be significantly higher than individual systems (p < 0.05). The proportions of lower brominated PBDEs congeners (mono- through tri-BDEs) were also higher in the combined system (Fig. 5.4).

6.6 Limitations and Further Study of the Research

6.6.1 Modification of TiO$_2$ and/or Exploration of Alternative

Photocatalyst Photocatalytic degradation of organic contaminants in water and wastewater is a promising technology and has been widely investigated in many studies (Lee et al., 2001). However, separation of the fine TiO$_2$ particles from solutions has become a challenge in the application of TiO$_2$ photocatalysis to wastewater treatment (Lee et al., 2001). For example, filtration was required to recover TiO$_2$ particles from effluent in the first phase of wastewater treatment (Chapter 5), which might increase the operational cost in commercial applications. Therefore, immobilization of TiO$_2$ as the catalyst in photocatalytic system was proposed but this is usually limited by the comparatively lower available external
Table 6.4 Comparison of removal efficiencies of BDE-209 by rice and reed in the combined system.

<table>
<thead>
<tr>
<th>Treatments</th>
<th>Initial BDE-209 concentrations (µg/L)</th>
<th>Removal efficiency of BDE-209 in the CWs (%)</th>
<th>Overall removal efficiency (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Unplanted</td>
<td>75</td>
<td>47.6 ± 7.47 b</td>
<td>73.1 ± 4.04 b</td>
</tr>
<tr>
<td>Unplanted</td>
<td>750</td>
<td>56.2 ± 4.14 b</td>
<td>71.6 ± 2.65 b</td>
</tr>
<tr>
<td>Rice</td>
<td>75</td>
<td>83.2 ± 5.24 a</td>
<td>92.7 ± 2.29 a</td>
</tr>
<tr>
<td>Rice</td>
<td>750</td>
<td>81.7 ± 2.50 a</td>
<td>91.7 ± 1.13 a</td>
</tr>
<tr>
<td>Reed</td>
<td>75</td>
<td>87.6 ± 4.13 a</td>
<td>94.6 ± 1.81 a</td>
</tr>
<tr>
<td>Reed</td>
<td>750</td>
<td>83.4 ± 2.35 a</td>
<td>92.5 ± 1.06 a</td>
</tr>
</tbody>
</table>

Removal efficiency of the CWs (%) was the percentage loss of BDE-209 in TiO₂ photocatalytically pre-treated wastewater, while the overall removal efficiency (%) was the percentage loss of BDE-209 of initial concentrations listed above. Values with the same small letter are not significantly different (p > 0.05) in the same column under one-way ANOVA test.
Table 6.5 Comparison of removal efficiencies of BDE-209 by different technologies

<table>
<thead>
<tr>
<th>Treatments</th>
<th>Initial BDE-209 concentrations (ug/L)</th>
<th>Removal efficiency of BDE-209 (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>TiO₂ photocatalysis</td>
<td>75</td>
<td>56.3 ± 5.78 c</td>
</tr>
<tr>
<td>TiO₂ photocatalysis</td>
<td>750</td>
<td>54.7 ± 9.47 cd</td>
</tr>
<tr>
<td>Hefengzhan, GI, pH 7</td>
<td>75</td>
<td>63.4 ± 0.326 b</td>
</tr>
<tr>
<td>Reed, GI, pH 7</td>
<td>75</td>
<td>48.1 ± 10.4 d</td>
</tr>
<tr>
<td>Combined system</td>
<td>75</td>
<td>93.6 ± 2.19 a</td>
</tr>
<tr>
<td>Combined system</td>
<td>750</td>
<td>92.1 ± 1.11 a</td>
</tr>
</tbody>
</table>

Values with the same small letter are not significantly different in the same column (p > 0.05) in the same column under one-way ANOVA test.
mass transfer area when compared with TiO\textsubscript{2} suspension and loss of catalyst particles during attrition (Balasubramanian et al., 2004). In order to extend the absorption spectra of these kinds of immobilized TiO\textsubscript{2} to visible light, some further modification, such as implantation of the Cr ion might be needed (Ao et al., 2007). Debromination of BDE-209 could also be achieved in aqueous solution by immobilization of other reductive reagents, such as nanoscale zerovalent iron (n-ZVI) on a cation-exchange resin (Li et al., 2007). Consequently, application of modified TiO\textsubscript{2} and alternative debromination assisting agents in the degradation of BDE-209 in the first phase of water treatment could be further investigated, in order to improve the reagents recovery process.

6.6.2 Field Trials of the Combined System

The public sewerage system in Hong Kong treated 2.69 million m\textsuperscript{3} of sewage every day of 2012 (DSD, 2012), and therefore the greenhouse-scale constructed wetland in the current study may not be able to accommodate and treat such a large quantity of sewage. Consequently, larger photocatalytic reactors and constructed wetlands on a large scale should be investigated to enhance the total volume available for treatments in the combined system and provide more information for future application in real sewage treatment.

6.6.3 Treatment of Mixed Contaminants in Wastewater
PBDEs were usually present in a mixture of organic contaminants, such as phthalates, alkylphenols, bisphenol A, PBDEs, PCBs and PAHs in wastewaters (Sánchez-Avila et al., 2009). These organic contaminants might interact with each other and the combined phytotoxicity of the contaminant mixture might affect the uptake of the contaminants in soil-plant system (Su and Zhu, 2006). It has been shown that the bioconcentration factor and plant transpiration rates declined due to the toxicity of mixed contaminants with a high concentration mixture of \(\alpha\)-Chlorophenol (20-100 mg/L) and 2,4-dichlorophenol (9-30 mg/L) (Su and Zhu, 2006). Therefore, in order to deal with the mixed contaminants in real wastewater, further investigation into the removal efficiency of mixed organic contaminants by the combined system might be required.

6.6.4 Exploration of Suitable Plants for the Constructed Wetlands

Rice (\textit{Oryza sativa} L.) and reed (\textit{Phragmites australis} L.) were chosen in the present study due to their promising ability in taking up various organic contaminants, including PBDEs (Du et al., 2013; Chu et al., 2006a). Furthermore, rice is the first crop to have its entire genome sequenced (International Rice Genome Sequencing Project, 2005). The current study can provide new information in transgenic technology for enhancement of PBDEs removal abilities of plants. Reed is a productive plant, which releases oxygen from its roots and
allows aerobic decomposition of organic matter and contaminants (Lee and Scholz, 2007). The oxygen leakage also supports the growth of AMF, and thus enhances the dissipation of PBDEs in soil (Tacon et al., 1983). Plants may exhibit diverse abilities in tolerating and treating different organic contaminants, and thus more wetland plants can be investigated in the future, in order to identify the most suitable plants (monoculture or polyculture) for treating the most common contaminants in sewage.

6.6.5 Enzymatic Debromination of PBDEs by Root Crude Enzyme

It has been reported that PBDEs can be transformed and debrominated to lower brominated PBDEs and hydroxylated-PBDEs by the root crude enzyme extracts from maize, pumpkin and ryegrass. Nitroreductase (NaR) and glutathione-transferase (GST) were suggested to be responsible for the in vitro phytodegradation of PBDEs (BDE-28, -47, -99 and -209) (Huang et al., 2013). In addition, the enzymatic degradation and transformation of o,p'-DDT, p,p'-DDT and PCBs were found to be more effective in reed than rice (Chu et al., 2006b). Therefore, enzymatic transformation of PBDEs by plant crude enzyme extracts should be investigated, in order to improve the efficiency of the combined system.

6.6.6 The transport Mechanism for the Uptake of Organic Contaminants by Other Plants

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Uptake and transport mechanisms for organic contaminants in plants can provide essential information for the assessment and recovery of contaminated sites (Chiou et al., 2001). Due to time limitation, the uptake and transport mechanisms of BDE-47, -99 and -209 were mainly focused on rice roots in the present study (Chapters 3 and 4). In order to facilitate the selection of wetland plants for particular organic contaminant (or mixtures) for the future application of the combined system, the mechanisms study needs to be extended to other parts of plants and species of plants. This will help to extend the database which is now confined mainly to rice and ryegrass (Su and Zhu, 2007).
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PUBLICATIONS

Journal Papers


Scientific Meetings Attended and Presentations

The 6th International Conference on Environmental Geochemistry in Tropics--Urban Issues. November 4-6, 2010, Institute of Urban Environment Chinese Academy of Sciences, Xiamen, China. (Poster presentation)

Croucher Advanced Study Institute – Remediation of Contaminated Land-Bioavailability and Health Risk. December 9-13, 2010, Hong Kong Baptist University, Hong Kong. (Poster presentation)

Area of Excellence (AoE) Annual Symposium. January 6-7, 2011. The University of Hong Kong. (Oral presentation)


CleanUp 2011 Conference (4th International Contaminated Site Remediation Conference). September 11-15, 2011, CRC for Contamination Assessment and
Remediation of the Environment, South Australia. (Poster presentation) – the Best Student Poster Award.

Area of Excellence (AoE) Annual Symposium. January 5-6, 2012. The University of Hong Kong. (Oral presentation)

Area of Excellence (AoE) Annual Symposium. January 10-11, 2013. The University of Hong Kong. (Oral presentation)

Awards

Best Student Poster Award, poster title: “Characterizing the Optimal Operation of Photocatalytic Degradation and Phytoremediation of BDE-209”, at the 4th International Contaminated Site Remediation Conference in Australia (September, 2011).

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November 2013