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Monitoring and visualizing of PAHs into mangrove plant by two-photon laser confocal scanning microscopy

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ABSTRACT

In this paper, we described the applications of two-photon laser confocal scanning microscopy (TPLCSM) for *in situ* monitoring and visualizing the localizations and movements of naphthalene, phenanthrene, and pyrene into living *Aegiceras corniculata* (L.) Blanco seedlings (*A. corniculata*). Experimental results demonstrated that all of the polycyclic aromatic hydrocarbons (PAHs) were observed entering into the root of *A. corniculata* and being transmitted to the stem. The transport processes and subsequent storages of the three typical PAHs into *A. corniculata* were similar. Further studies indicated that the transmission rates of the PAHs in *A. corniculata* were in the order of naphthalene > phenanthrene > pyrene. Compared with the control group, the growth of the *A. corniculata* was inhibited by these three specific PAHs, and the inbibitional effect of naphthalene was the most obvious ($P < 0.05$). Furthermore, without the need for sample manipulation or modification this TPLCSM provides us a real-time tool for direct observation of organic chemicals within plants.

1. Introduction

PAHs are known to be widespread hazardous pollutants of great environmental concern, which are known to be teratogenic, carcinogenic and mutagenic (Gorshkov, 2008; Laender et al., 2011). There are many routes by which PAHs from diverse sources reach the environment, such as incomplete combustion of fossil fuels, waste incineration, vehicle exhausts, industrial processes, urban runoff, petroleum spillage and atmospheric fallout (Augusto et al., 2010). The persistence of PAHs in the environment might be harmful to humans and ecosystems due to their bioaccumulation and biomagnification via food chains (Bayen et al., 2005; Ke et al., 2002). In addition, PAHs are lipophilic, which might make them be accumulated in vegetation. Some research suggests that the environmental fate of PAHs could be affected by their uptake to vegetation from the air and soil. Moreover, the subsequent processing, including storage, transport, photodegration, metabolism or revolatilization should also be studied (Wu et al., 2009).

Mangrove forests mainly found on the marine alluvium along the coastlines of tropical and sub-tropical regions, are often under pollution stress caused by various anthropogenic pollutants. With its unique features of high primary productivity, abundant detritus, rich organic carbon and anoxic conditions, the mangrove ecosystems are considered to be reservoir of lipophilic contaminations, including PAHs from various sources (Rieumont et al., 2012; Loi et al., 2011; Chang et al., 2009). The environmental behaviors of PAHs on mangroves are thought to be potential key component in the global cycling of PAHs (Brito et al., 2009). Many studies also illustrate that PAHs could be accumulated into the different tissues of many mangrove species (Lu et al., 2005; Tian et al., 2008; Santos et al., 2011). However, the traditional methods utilizing the entirely destructive separation and extraction procedures might destroy the originally existing forms and eliminate the spatial distributions of PAHs in/on different tissues of mangrove plants. Thus, results derived from these traditional methods only reflect the total concentrations of PAHs in the whole leaves, stems, or roots, and it is difficult to understand where the PAHs within the plants are located, how the PAHs reach those locations and how the PAHs are processed within these compartments. Therefore, an *in situ* approach for direct investigation of the uptake, translocations and sequent locations of PAHs in the mangrove plants should be studied, which might play an important role in the environmental fate of PAHs and a study on the phytoremedation of mangrove plants.

Wild and his co-workers use the TPLCSM to observe the locations of anthracene in living maize leaves and track the uptake and storages of anthracene and phenanthrene in the roots of maize and wheat (Wild et al., 2004, 2005). In our previous studies the TPLCSM was utilized for *in situ* visualization of three specific PAHs into viviparous hypocotyls of *Kandelia obovata* seedlings (*K. obovata*) of salt resistance mangrove species (Wang et al., 2010). While

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the *Aegiceras corniculata* is the typical salt-tolerant mangrove plant which is the non-viviparous species. How are the uptake, movements and localizations of the typical PAHs within the living *A. corniculata*? Are the pathways of the PAHs entering into *A. corniculata* different with the *K. obovata*? These questions are greatly significant for us to know more about the environmental fate of the PAHs into the inner tissues of mangrove plants. Thus, this study extended the applications of TPLCSM to monitor the uptake, transportations and processes of three specific PAHs from growth medium into the roots of *A. corniculata* and to understand exactly their subsequent storages within the root, stem and leaf, during the 19-day growth study. Moreover, plenty of studies show that the surfactants weak toxicity to plant are always used to enhance the solubilization and mobilization of PAHs in water thereby increase the bioconcentration factors of PAHs accumulation in the plant tissues (Wang et al., 2010; Gao et al., 2006; Li et al., 2001). Thus the Tween 80 as a non-ionic surfactant was used in this study.

2. Materials and methods

2.1. Apparatus and compounds generation

TPLCSM detections of naphthalene, phenanthrene and pyrene into *A. corniculata* were carried out with a Leica TCS-SP2 AOBS scanning system equipped with a Tsunami Ti: Sapphire Femtosecond, Direct-Mirror Coupling and a Leica DM IRE2 inverted microscope. The laser wavelength of the TPLCSM was set at 780 nm. Images of the three specific PAHs in/on the tissues of *A. corniculata* were collected and processed using the Leica TCS-SP2 imaging software, respectively (Wang et al., 2010).

The naphthalene, phenanthrene and pyrene (purity > 99%) were obtained from Sigma Aldrich (USA) without further purification. The stock solution of each PAH compound was prepared by dissolving the solutes in acetone, respectively. All the solutions were stored in the brown volumetric flask at 4 °C and wrapped with aluminum folds to avoid possible photodegradation. Working solutions of the three specific PAHs were prepared by dilution of the stock solution in acetone before use. The culture solutions were prepared with half-strength Hoagland solution containing 1 mg L^{-1} of each PAH and 104.8 mg L^{-1} of Tween 80, respectively (Wang et al., 2010; Burken and Schnoor, 1997).

2.2. Plant preparation

The two-month-old *A. corniculata* collected from mature mangrove trees growing in Cao Putou village, Longhai city, China (longitude: 24°54′ latitude: 117°23′ altitude: 0 m above sea level), were cultivated hydroponically in sand bed. Under a 12 h photoperiod, the plants were illuminated by 400 W sodium Lamp and the green house temperature was maintained at 25–28 °C. Plants with similar length and fresh weight, which were about 57–61-day-old were utilized in the experiments. Five plants of *A. corniculata* were cultured in glass containers, with triplicated for each treatment. The containers with the drilled holes on their cap were covered by aluminum foil to avoid any possible photodegradation or volatilization of the PAHs. Both the roots and part of stems of *A. corniculata* were immersed into the culture solutions contaminated with different PAHs. The lost solutions in all containers in the course of experiment were replenished with the distilled water to keep the same volume. The tissues obtained from the living *A. corniculata* contaminated with different PAHs were studied over a 19-day period. Replicate experiments were conducted.

3. Results and discussion

3.1. Effects of the three specific PAHs on the growth of Ac

Henner found that low molecule of PAH (<3 rings) has strong phytotoxicity to the plant, while the high molecule of PAH (>3 rings) has no phytotoxicity (Henner et al., 1999). Lu revealed that as the time processed, the inhibition of pyrene to the growth of *K. obovata* was obvious (Lu, 2002). In the experiment, the effects of three specific PAHs on the growth of *A. corniculata* were also studied. It turned out that the effects of the three specific PAHs on the growth of leaves, stems and roots of *A. corniculata* were obviously observed after cultivated in contaminated solutions for 19 days (Table 1). The experimental results revealed that compared with the control group (0 mg L^{-1}), the biomass of *A. corniculata* decreased under the pollution of each PAH. It was also from Table 1 that under the pollution of the naphthalene and phenanthrene, the average length of roots, stems and leaves was decreased more significantly than that in control group, which were reduced by 21.4% and 14.3% ($P < 0.05$), respectively. However, there was no obvious difference under the pyrene stress ($P > 0.05$). While the root numbers of each plant were reduced by 41.7%, 29.2% and 16.7% in the pollution presence of naphthalene, phenanthrene and pyrene, respectively, which showed significant difference with the control group ($P < 0.05$). Table 1 also showed that the effects of naphthalene, phenanthrene and pyrene on the height and average length of the shoots were not obvious compared with the control group ($P > 0.05$). In conclusion, the growth of the *A. corniculata* was inhibited by the three typical PAHs, and the inbibitional effect of naphthalene was the most obvious. The reason might be that the *A. corniculata* has strong accumulation of the naphthalene, or the naphthalene could cause great phytoxicity to this mangrove species (Oheim et al., 2001).

3.2. Study on the storage and transfer of PAHs into the A. corniculata

Root absorption from contaminated soil or water has been demonstrated to be one of the most important pathways for a variety of organic chemicals, including PAHs (Wang et al., 2010; Kang et al., 2010; Lohmann et al., 2011). It is recognized that the PAHs adsorbed onto the mangrove plants mainly accumulate into the roots or trunks, from where the contaminants are not easily transferred into the ground tissues. Therefore, the amount of pollutants existing within the leaves is very little (Lu, 2002; Tam et al., 2001; Tam and Wong, 2008). In this section, coupled with the autofluores-

Table 1
Effect of different kinds of PAHs on the growth of root, stem, leaf and height growth of *A. corniculata* in water culture (19 days).

PAHs concentration (mg L^{-1})		Leaf length (cm)	Stem length (cm)	Root length (cm)	Root number	Height (cm)	Number of plant
NAP	0	2.5 ± 0.6	8.0 ± 2.2	5.6 ± 3.5	24 ± 3	16.0 ± 3.3	5
	1	2.0 ± 0.8	7.5 ± 1.4	4.4 ± 2.8	14 ± 6	13.8 ± 4.5	15
PHE	0	2.5 ± 0.6	8.0 ± 2.2	5.6 ± 3.5	24 ± 3	16.0 ± 3.3	5
	1	2.2 ± 0.6	7.8 ± 1.9	4.8 ± 2.4	17 ± 4	15.2 ± 2.3	15
PYR	0	2.5 ± 0.6	8.0 ± 2.2	5.6 ± 3.5	24 ± 3	16.0 ± 3.3	5
	1	2.1 ± 0.4	8.1 ± 2.0	5.1 ± 3.7	20 ± 5	16.3 ± 2.1	15