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In situ simultaneous determination the photolysis of multi-component PAHs adsorbed on the leaf surfaces of living *Kandelia candel* seedlings

Li Chen, Yong Zhang*, Beibei Liu

State Key Laboratory of Marine Environmental Science (Xiamen University), Environmental Science Research Center, Xiamen University, Xiamen 361005, Fujian Province, China

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ABSTRACT

A fiber-optic fluorimetry for *in situ* simultaneous determination of fluorine (Flu), phenanthrene (Phe) and fluoranthene (Fla) adsorbed on the leaf surfaces of *Kandelia candel* (*Kc*) seedlings was developed. Experimental results showed that the linear ranges for determination of Flu, Phe and Fla adsorbed on *Kc* leaves were 35–700, 5–900 and 2–450 ng/spot, respectively. The detection limits for Flu, Phe and Fla were 9.11, 1.65 and 0.90 ng/spot and with the relative standard deviations less than 10.32%, 7.56% and 4.29% ($n = 9$), respectively. The recovery results for Flu, Phe and Fla adsorbed on *Kc* leaves were 83.0–91.2, 78.5–88.5 and 91.5–107.3%, respectively. Under the laboratory experimental conditions, the photolysis processes of Flu, Phe and Fla individual and in mixtures adsorbed on the leaf surfaces of living *Kc* seedlings were studied. Results showed that the photolysis of Flu, Phe and Fla individual and in mixtures adsorbed on the leaf surfaces of *Kc* seedlings followed first-order kinetics with photolysis rates in the order of Flu > Fla > Phe on the *Kc* leaves. An antagonistic effect was found when the three polycyclic aromatic hydrocarbons (PAHs) were co-adsorbed on living *Kc* seedlings. The experimental results also indicated that photolysis was the main transformation pathway for Flu, Phe and Fla both individual and in mixtures adsorbed on *Kc* leaves, whereas disappearance of the adsorbed Flu, Phe and Fla as a result of volatilization and leaf absorption could be negligible during the experimental period.

1. Introduction

Polycyclic aromatic hydrocarbons (PAHs), are known to be widespread hazardous pollutants, produced via natural and anthropogenic sources, mainly generated during the incomplete combustion of solid and liquid fuels or derived from industrial activities. PAHs are ubiquitous in different natural phases such as plant, soil, sediment, water and air, and are extremely harmful to ecosystems and health of humans due to their high degree of teratogenicity, mutagenicity and carcinogenicity [1–3]. Most PAHs in the environment are hydrophobic and their low water solubility limits their biodegradation in the environment [4–6]. Recently, many chemical technologies for the degradation of PAHs have been paid much attention, and among which the photolysis has been greatly emphasized [7]. And some studies have also experimentally shown that photolysis is an important abiotic transformation pathway for most PAHs in the environment [8,9].

Leaf surfaces are covered with a complex lipid cuticle that can adsorb hydrophobic organic pollutants from the atmosphere, and the larger surface area and more leaf-wax content a leaf is the more PAHs it can adsorb [10–12]. As a result, it is commonly believed that the adsorption of PAHs by leaves is the most important pathway by which they become enriched in vegetations [10,13]. That implies that behaviors of PAHs adsorbed on leaf surfaces play a significant role in the migration of PAHs from air to earth's surface and from air to food chain. Unfortunately, up to now, because of the lack of *in situ* research methods, current researches in this area are rarely carried out. Thus, it is very necessary to establish an *in situ* method to discuss the environmental behaviors of PAHs adsorbed on plant leaves.

The mangrove ecosystem, a predominantly intertidal estuarine wetland, nowadays, is exposed to anthropogenic contamination which bring many pollutants, such as, heavy metals, organochlorine pesticides, polychlorinated biphenyls, PAHs and so on [10,14–18]. Mangrove forest is important to humans for a variety of reasons, including aquaculture, agriculture, forestry and protection against shoreline erosion. Meanwhile, mangrove forest can provide us with firewood, building material, other local subsistence use and a source of various food categories, which become the main contributors to human intake of PAHs [19,20]. Therefore, recently, much attention has been focused on the researches of mangrove pollution ecology [21–24]. Mangrove leaves, in order to adapt to their special ecological habit, are common with large surface areas and thick lipid cuticle. In other words, theoretically, mangrove leaves have a great potential to adsorb atmospheric PAHs. Simonich and Hites

* Corresponding author. Tel.: +86 592 2188685/18959288685; fax: +86 592 2184977.
E-mail address: yzhang@xmu.edu.cn (Y. Zhang).

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have developed a mass-balance model for measuring the amount of the PAHs from the atmosphere, and their results showed that 44 ± 18% of the PAHs emitted into the atmosphere were removed by plant leaves in the northeast of the United States [25]. However, after being absorbed by plant leaves, what are the final environmental behaviors of them are still unknown. Therefore, it is of great necessary to study the fate of PAHs after being adsorbed on plant leaves to understand more about the environmental behaviors of them.

Recently, environmental behaviors of PAHs on spruce or pine needles have been studied. And the experimental results showed that photodegradation of PAHs on the surface of the leaves played an important role in the environmental fate of PAHs [26–28]. In this paper, *Kandelia candel* (*Kc*), one of the most widespread mangrove species in China was used for the experiment. Obviously, the leaf morphology of *Kc* is quite different from spruce and pine needles, hence, the results they obtained whether would be suitable to *Kc* leaves is still unknown. On the other hand, all of the determination method used in these published works was GC/MS. Though it is with high precision and accuracy, it needs an extraction separation step before sample analysis that is not only arduous, but also causes secondary pollution problems because of the organic solvent used. At the same time, all of these traditional methods utilizing entirely destructive chemical extraction techniques might destroy the originally existing forms and eliminate the spatial distribution of PAHs on/in the leaves. Meanwhile PAHs often exist as a mixture in the environment, previous researches mainly centered on single component PAH adsorbed on plant leaves and rarely discussed the interaction of PAHs from each other in mixtures. Recently, photolysis of single PAH adsorbed on the surfaces of mangrove leaves, such as anthracene (An) [29,30] and fluoranthene (Fla) [31] has been investigated by fluorimetry in our laboratory, but *in situ* determination of multi-components PAHs adsorbed on living mangrove seedlings has never been reported. Thus, it is essential to establish an *in situ* method to further investigate the environmental behaviors of multi-component PAHs adsorbed on the leaf surface of *Kc* seedlings, and then to understand more of the fate processes and mechanism of PAHs adsorbed on *Kc* leaf surface.

In this work, based on our previous work [29–31] and the method presented in reference [32], fluorene (Flu), phenanthrene (Phe) and Fla were selected as model PAHs compounds. A fiber-optic fluorimetry was employed to *in situ* simultaneous determination of Flu, Phe and Fla individual and in mixtures adsorbed on leaf surfaces of living *Kc* seedlings, because of the great advantages of fiber-optic fluorimetry, such as high light focalization, low weight and small size, suitable for on-line, *in situ*, real time and remote detection of pollutants [33]. To make sure whether PAHs adsorbed on mangrove leaves could be photolyzed or not, and furthermore, to investigate the photolysis processes and mechanism of the three PAHs individual and in mixtures adsorbed on leaf surfaces of *Kc* seedlings.

2. Materials and methods

2.1. Apparatus and reagents

All of the fluorescence spectra were obtained on a Cary Eclipse fluorescence spectrophotometer equipped with a 150 W Xenon flash lamp and fiber optic accessories (Varian, USA). The spectrofluorimeter was controlled by Cary Eclipse software for acquiring and processing the spectral data. Instrumental parameters were as follows: excitation and emission slits were set at 20 and 10 nm; scan speed was 600 nm min^{-1}; PMT voltage was 600 V. The UV–vis spectra of the three PAHs were scanned by a UV–vis spectrophotometer (Varian, USA). A CHF-XM 500 W with a high pressure mercury lamp (including fiber optic) (Beijing Trusttech, Co., Ltd., China) was used as light source for the photolysis experiment. A ZDS-10 illuminometer (Shanghai Jiading Xuelian Instrument Factory, China) was used to measure the intensity of the light source during the experiment. A 10 μL flat head micro-injectors (Shanghai Medical Laser Instrument Plant, China) was used to introduce PAHs solution onto *Kc* leaf surfaces.

To prepare PAH stock solutions, 0.2000 g solid Flu (Alfa Aesar, USA, purity ≥98%), 0.2000 g Phe (Alfa Aesar, USA, purity ≥98%) and 0.2000 g Fla (Acros, Belgium, purity ≥98%), which were used without any purification, were separately dissolved in 100 mL acetone in brown volumetric flasks and stored at 4 °C to avoid possible photolysis. Working solutions of Flu, Phe and Fla, individual and in mixtures were prepared by transferring small aliquots of each stock solution into several 10 mL colorimetric tubes, and then acetone solution was added to the mark to obtain their calibration curves and to study the photodegradation processes of them adsorbed on the leaf surfaces of living *Kc* seedlings.

2.2. Sample collection

Mangrove hypocotyls of *Kc* were collected from Longhai mangrove reserve located in Zhangzhou, Fujian, China (east longitude: 117°29′–118°14′; north latitude: 24°11′–24°36′; altitude: 0 m above sea level). *Kc* hypocotyls of about the same size and maturity were collected and quickly taken to laboratory for cultivation. *Kc* seedlings were cultivated with sediments which were sampled from mangrove forest for about twenty-four months. Then *Kc* seedlings of about the same height (55 ± 0.5 cm) were used for the following experiments.

2.3. Pretreatment of Kc seedlings for the experiment

Three living *Kc* seedlings of about the same height were chosen for the photolysis experiment of the three PAHs. Three leaves of about the same size were chosen from these *Kc* seedlings, and one leaf was selected from each *Kc* seedling. All of the selected leaves were carefully rinsed with tap water and Milli-Q water three times, respectively. After air-drying, the determination areas on the leaf surface were selected and marked on these areas by using the large end of a 5 mL pipette. Three determination points for each part ($n = 9$) were made as shown in Fig. 1. The area of the circle is defined as a unit of 'spot' which had a same size produced by the fiber optical probe. Then the three PAHs individual and in mixtures dissolved in acetone solution were introduced onto these 'spots' using a 10 μL flat head micro-injector at room temperature.

2.4. Determination of PAHs adsorbed on the leaf surface of living Kc seedlings

The amounts of the three PAHs adsorbed on the *Kc* leaves were directly determined by a Cary Eclipse fluorescence spectrophotometer equipped with fiber optic accessories. Living *Kc* seedlings with the PAHs adsorbed on their leaf surfaces were put under the optical fiber probe, and the leaves were kept smooth during the experiment (Fig. 1). Three-dimensional spectra of the three PAHs individually adsorbed on the *Kc* leaf surfaces were scanned by fiber-optic fluorimetry. The data obtained were analyzed and processed as mentioned in reference [32] to choose the optimized maximum excitation and emission wavelengths. The spectrofluorimeter was controlled by Cary Eclipse software for acquiring and processing the spectral data. With such software, fluorescence intensities of the three PAHs in mixtures can be obtained in one scan, since optimized wavelength for each of them had already been set up before scanning. The determination wavelengths for Flu, Phe and Fla were λ_{ex} = 270 nm, λ_{em} = 309 nm; λ_{ex} = 253 nm, λ_{em} = 367 nm;

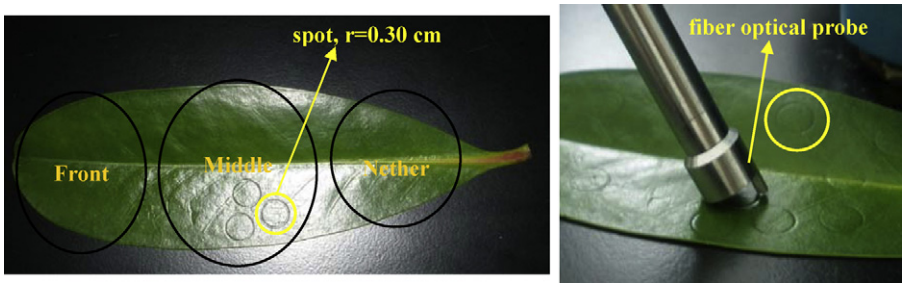

Fig. 1. Schematic diagram for the determination areas and the method for the measurement of PAHs adsorbed on the leaf surface of *Kc* seedlings.

and λ_{ex} = 358 nm, λ_{em} = 466 nm, respectively. It only took less than 1 min to obtain the relative fluorescence intensities of the three PAHs in mixtures.

Serial concentrations of Flu–acetone, with fixed concentrations of Phe and Fla were introduced onto the leaf surfaces of *Kc* seedlings using a 10 μL flat head micro-injector, and after volatilization of the acetone from the sample spots on the *Kc* leaves, the relative fluorescence intensities of Flu, Phe and Fla adsorbed on *Kc* leaf surfaces were directly determined using their optimized determination wavelengths. The same operations were repeated nine times, respectively. Average means of the nine measurements were used to describe the final results, and the relative standard deviations were also calculated. Serial concentrations of Phe or Fla, with the fixed other two PAHs were also prepared and determined with the same approach as Flu.

2.5. Photolysis of the three PAHs adsorbed on the living Kc seedlings leaf surface

Using a high pressure mercury lamp as the light source (including optical fiber), the photolysis processes of the three PAHs individual and in mixtures adsorbed on the living *Kc* seedlings leaf surface were investigated. The initial concentrations of Flu, Phe and Fla were 400, 500 and 200 ng/spot, respectively, which were all at the middle of their concentration linear dynamic ranges. In order to keep the emit light intensity steady during the experiment, the mercury lamp must be preheated for half an hour before starting the experiment. The light intensity was controlled by adjusting the height between the *Kc* leaf surfaces and the optical fiber probe. The illumination intensity on the *Kc* leaf surfaces, the temperature and relative humidity during the photolysis experiment were $8.86(\pm0.05)\times10^4$ lx, 25 ± 0.2 °C and 70 ± 4.0%, respectively.

The tested leaves were put and kept smoothly under the spot of light that was guided by an optical fiber to avoid heating effects from the mercury lamp on photolysis processes and to make sure that the same light intensity were emitted to all PAHs adsorbed on them during the experiment. After a certain interval, the relative fluorescence intensities of the three PAHs were detected by using the fiber-optic fluorimetry. Volatilization and absorption of the three PAHs adsorbed on the leaves surface were also investigated to explain the disappearance of the PAHs adsorbed on the leaf surfaces. Therefore, control experiments were carried out by keeping the *Kc* seedlings in darkness, and then the relative fluorescence intensities of the three PAHs adsorbed on *Kc* leaves were also determined after the same time interval as those of photolysis experiments.

2.6. UV–vis spectrum of the three PAHs dissolved in water

It has been reported that the UV–vis spectra show the exact absorption characteristic of the target compounds which are very important for the explanation of the different photolysis results [26–28]. Therefore, certain amounts of the three individual PAH–acetone solutions were transferred into several colorimetric tubes. After allowing evaporation of the solvent by a gentle flow of high-purity nitrogen gas (≥99.9%), Milli-Q water was added to the mark of the colorimetric tubes. And then these tubes were ultrasonicated for 30 min at room temperature, kept them in dark for 5 h to ensure that the target PAHs were sufficiently dissolved, as well as to avoid possible photolysis. Finally, their UV–vis spectra were obtained by a UV–vis spectrophotometer.

3. Results and discussion

3.1. Theoretical contour map and fluorescence spectra of Flu, Phe and Fla adsorbed on the leaf surfaces of Kc seedling

The optimal determination wavelengths of the three PAHs should firstly be chosen for the direct determination of them. And this is also the precondition for the accurate determination of the three PAHs adsorbed on the leaf surfaces of *Kc* seedlings [33,34]. Therefore, three-dimensional spectra of the three PAHs individually adsorbed on the leaf surfaces of living *Kc* seedlings were scanned and shown in Fig. 2. As can be seen from Fig. 2, three 'measurement points (A, B and C)', without fluorescence interference to each other for Flu, Phe and Fla can be picked out, respectively. The determination wavelengths for Flu, Phe and Fla were chosen as: A: λ_{ex} = 270 nm, λ_{em} = 309 nm; B: λ_{ex} = 253 nm, λ_{em} = 367 nm; and C: λ_{ex} = 358 nm, λ_{em} = 466 nm. Based on the experimental results obtained by conventional fluorimetry, as shown in Fig. 3, with the accurate excitation and emission wavelengths for Flu, Phe and Fla, the fluorescence intensities of the three PAHs in mixtures adsorbed on the leaf surfaces of *Kc* seedlings showed a very little difference compared to that of the three of them in individual. The fluorescence intensities of the three PAHs in mixtures were almost the same (such as Fla) or a bit less (such as Flu and Phe) than that

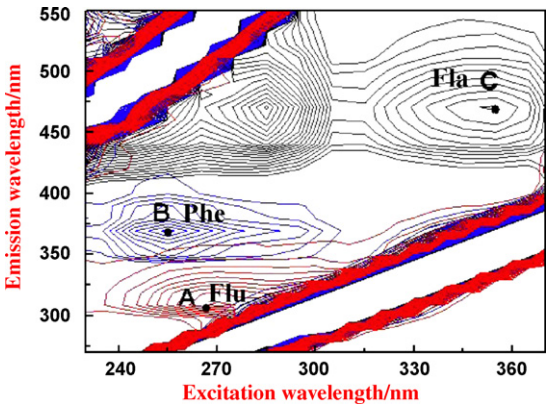

Fig. 2. Theoretical contour map of Flu, Phe and Fla and their measurement points. Concentrations of Flu, Phe and Fla were all 400 ng/spot.

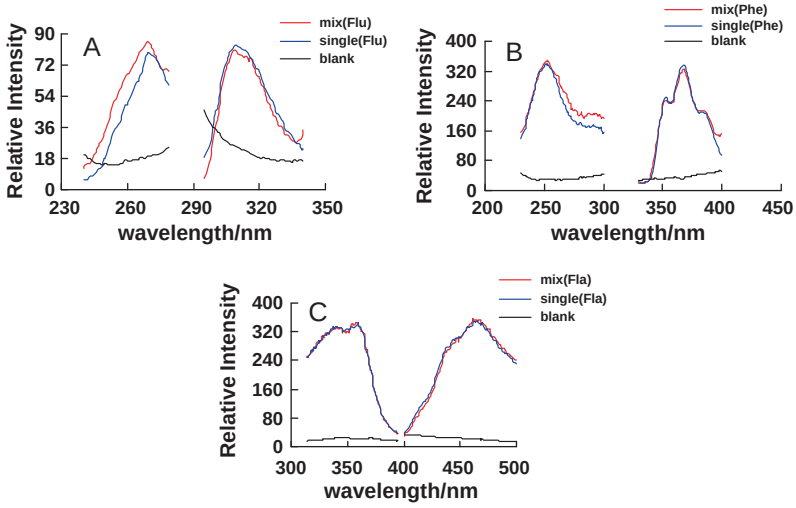

Fig. 3. Fluorescence excitation and emission spectra as individual component and in mixtures. (A) Flu (400 ng/spot, λ_{ex} = 270 nm, λ_{em} = 309 nm). (B) Phe (500 ng/spot, λ_{ex} = 253 nm, λ_{em} = 367 nm). (C) Fla (200 ng/spot, λ_{ex} = 358 nm, λ_{em} = 466 nm).

Table 1
Merits of the established method.

PAHs	Linear regression equation	Linearity range (ng/spot)	RSD% (n = 9)	Coefficient relevant	Detection limit[c] (ng/spot)	Concentration of other contents (ng/spot)
Flu	$y^{b} = 0.207x^{a} - 1.16$	35–700	10.32	0.9950	9.11	Phe: 500Fla: 200
Phe	$y = 0.627x + 18.6$	5–900	7.56	0.9948	1.65	Flu: 400 Fla: 200
Fla	$y = 1.87x - 23.2$	2–450	4.29	0.9844	0.90	Flu: 400Phe: 500

[a] x: concentration of PAHs.
[b] y: fluorescence intensities of PAHs adsorbed on the leaves of *Kc* seedlings.
[c] The detection limit was calculated using 3Sb/K, where Sb stands for the standard deviation of the blank (n = 9), K stands for the slope of each calibration curve.

Table 2
Results of interference experiments for the three PAHs adsorbed on the surfaces of *Kc* leaves(n = 9).

No.		1	2	3	4	5	6	7	8	9
Flu (ng/spot)	Added	400.0	400.0	400.0	400.0	400.0	400.0	400.0	400.0	400.0
	Found	396.0	400.3	390.9	392.5	392.8	379.2	394.5	390.9	399.7
Phe (ng/spot)	Added	50.0	50.0	50.0	400.0	400.0	400.0	900.0	900.0	900.0
	Found	49.4	48.6	53.9	386.0	398.3	384.8	892.4	879.3	888.7
Fla (ng/spot)	Added	15.0	200.0	400.0	15.0	200.0	400.0	15.0	200.0	400.0
	Found	16.1	194.8	394.1	16.6	193.8	396.6	16.0	197.8	404.3
No.		10	11	12	13	14	15	16	17	18
Flu (ng/spot)	Added	50.0	50.0	50.0	300.0	300.0	300.0	600.0	600.0	600.0
	Found	47.2	51.2	44.8	306.6	298.1	285.1	565.3	562.7	555.7
Phe (ng/spot)	Added	500.0	500.0	500.0	500.0	500.0	500.0	500.0	500.0	500.0
	Found	497.7	487.3	484.5	494.9	490.9	473.0	477.7	475.8	470.1
Fla (ng/spot)	Added	15.0	200.0	400.0	15.0	200.0	400.0	15.0	200.0	400.0
	Found	14.7	201.5	392.5	15.9	200.9	394.3	16.0	195.5	388.2
No.		19	20	21	22	23	24	25	26	27
Flu (ng/spot)	Added	50.0	50.0	50.0	300.0	300.0	300.0	600.0	600.0	600.0
	Found	45.7	44.1	48.4	290.9	276.4	278.5	585.4	569.8	569.4
Phe (ng/spot)	Added	50.0	400.0	900.0	50.0	400.0	900.0	50.0	400.0	900.0
	Found	39.0	376.9	875.3	58.1	374.9	858.9	35.1	374.5	874.2
Fla (ng/spot)	Added	200.0	200.0	200.0	200.0	200.0	200.0	200.0	200.0	200.0
	Found	195.5	193.7	198.0	197.0	189.6	195.7	196.7	190.8	194.5

Table 3
Results of recovery experiments of Flu, Phe and Fla adsorbed on the surface of *Kc* leaves (n = 9).

No.	Flu			Phe			Fla		
	Added (ng/spot)	Found (ng/spot)	Recovery (%)	Added (ng/spot)	Found (ng/spot)	Recovery (%)	Added (ng/spot)	Found (ng/spot)	Recovery (%)
1	50	45.6	91.2	50	39.3	78.6	50	53.7	107.3
2	100	83.1	83.1	200	163.6	81.8	100	94.1	94.1
3	300	266.4	88.8	400	354.0	88.5	200	182.9	91.5

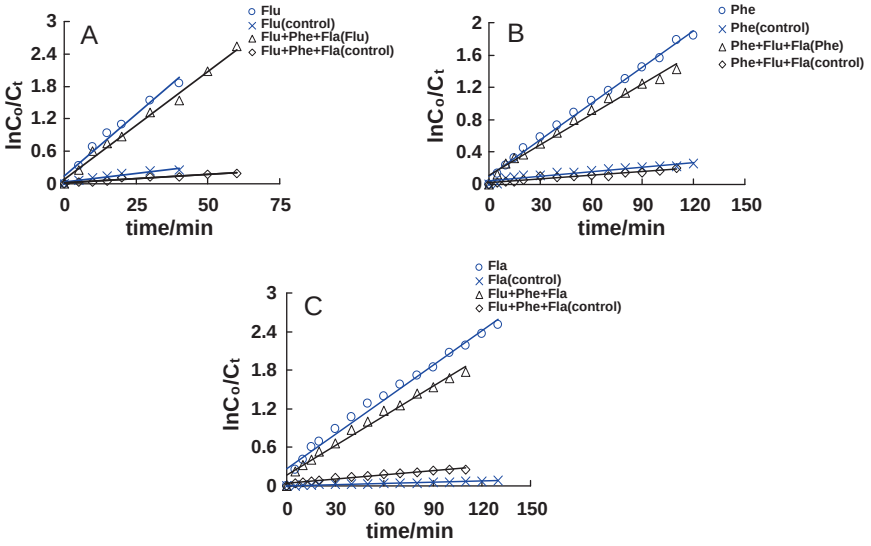

Fig. 4. Photolysis processes of the Flu (A), Phe (B), Fla (C) individual and in mixtures adsorbed on the upper leaf surface of *Kc* seedlings. The concentrations of Flu, Phe and Fla were 400, 500 and 200 ng/spot, respectively.

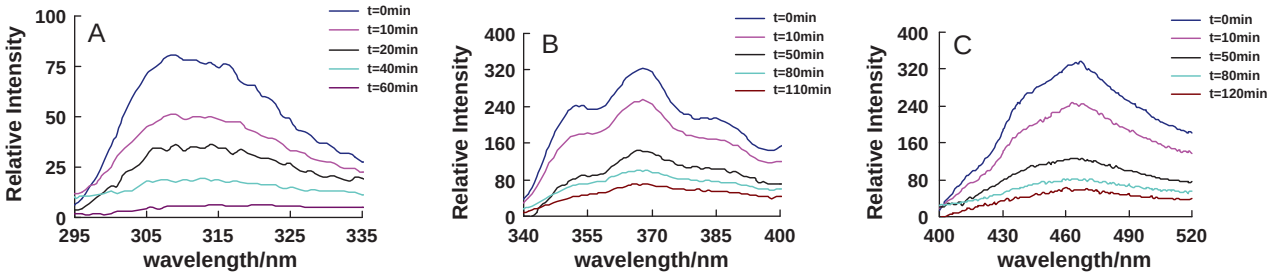

Fig. 5. The fluorescence spectra of the three PAHs in mixtures (A: Flu, B: Phe and C: Fla) during the photolysis periods. The initial concentrations of Flu, Phe and Fla were 400, 500 and 200 ng/spot, respectively.

Table 4
The kinetic parameters for the disappearance of the three PAHs individual and in mixtures adsorbed on the upper leaf surfaces of *Kc* seedlings ($n = 9$).

	k_T (min^{-1})	R_T^2	k_C (min^{-1})	R_C^2	k_P (min^{-1})	$t_{1/2}$ (photolysis) (min)
PAHs						
Flu	0.0519	0.9739	0.0063	0.9243	0.0456	15.2
Phe	0.0169	0.9920	0.0018	0.9149	0.0151	45.9
Fla	0.0185	0.9849	0.0006	0.9799	0.0179	38.7
Flu + Phe + Fla						
Flu	0.0431	0.9921	0.0031	0.9370	0.0400	17.3
Phe	0.0142	0.9876	0.0015	0.9310	0.0127	54.6
Fla	0.0177	0.9840	0.0022	0.9622	0.0155	44.7

k_C: the disappearance rate constants of PAHs in the control experiments. k_T: the total disappearance rate constants. k_P: the photolysis rate constant. R_T^2: a measure of the goodness of fit for the total illumination period. R_C^2: a measure of the goodness of fit for the control experiments. $t_{1/2}$: the half-lives of the three PAHs individual and in mixtures adsorbed on the leaf surfaces of *Kc* seedlings.

Table 5
Changes in the amount of the three PAHs individual and in mixtures adsorbed on the leaf surfaces of *Kc* seedlings ($n = 9$).

	C_0 (ng/spot)	C_F (ng/spot)	ΔC_T (ng/spot)	C_{CF} (ng/spot)	ΔC_C (ng/spot)	ΔC_P (ng/spot)	$\Delta C_P/C_0$ (%)	$\Delta C_C/C_0$ (%)
PAHs								
Flu	400	52.4	347.6	315.5	84.5	263.1	65.8	21.1
Phe	500	44.5	455.5	379.8	120.2	335.3	67.1	24.0
Fla	200	25.3	174.7	186.1	13.9	160.8	80.4	6.95
Flu + Phe + Fla								
Flu	400	31.9	368.1	327.5	72.5	295.6	73.9	18.1
Phe	500	61.8	438.2	402.7	97.3	340.9	68.2	19.5
Fla	200	41.8	158.2	240.6	40.6	117.6	58.8	20.3

C_F stands for the final amount of the three PAHs adsorbed on the leaf surface of *Kc* seedlings at the end of photolysis experiment. ΔC_T stands for the total disappearance amount of the three PAHs at the end of photolysis experiment, $\Delta C_T = C_0 - C_F$. C_{CF} stands for the final amount of the three PAHs adsorbed on the leaf surface of *Kc* seedlings for the control experiments. ΔC_C stands for the disappearance amount of the three PAHs for the control experiments, $\Delta C_C = C_0 - C_{CF}$. ΔC_p stands for the amount of the three PAHs disappearance by photolysis, $\Delta C_P = \Delta C_T - \Delta C_C$.

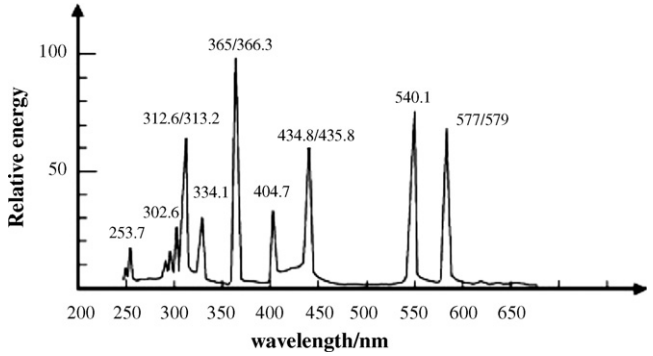

Fig. 6. Energy distributed curve of high pressure mercury lamp used in the experiment.

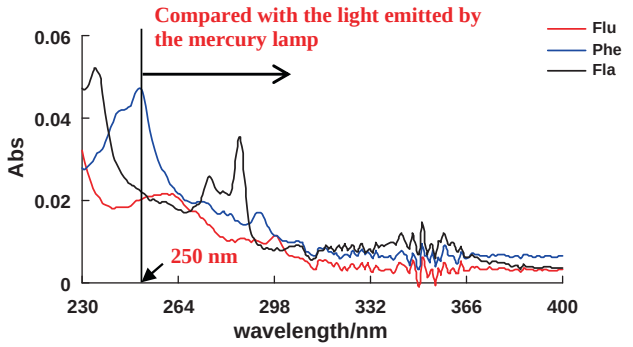

Fig. 7. UV–vis spectra of Flu, Phe and Fla individual dissolved in Milli-Q water. The concentration of Flu, Phe and Fla were all 0.2 mg/L.

of the three PAHs individually adsorbed on the leaf surfaces of *Kc* seedlings. It also can be seen from Fig. 3 that the autofluorescence of the uncontaminated *Kc* leaves was too weak to interfere the determination of Fla and Phe. As for Flu, the autofluorescence of the uncontaminated *Kc* leaves had a little interference. However, Flu adsorbed on *Kc* leaves can still be quantitively determined. Also from Fig. 3, it can be seen that there was no interference to the background value of *Kc* leaves as acetone used as blank as presented in reference [31]. It is implied that the three PAHs in mixtures adsorbed on the leaf surfaces of *Kc* seedlings could be simultaneously determined by fluorimetry.

3.2. Analytical merits of the established method

As we known that the amount of PAHs adsorbed on plant leaves were very small in the natural environment, in order to know the exact amount change of them during the photolysis process, accurately quantifying the amount of PAHs adsorbed on the leaf surfaces of *Kc* seedlings is a prerequisite for the following photolysis experiments. Therefore, a series of Flu–acetone concentrations with fixed concentrations of Phe and Fla was prepared according to the method mentioned in reference [33]. Then they were introduced onto the tested leaf surfaces, and the relative fluorescence intensities of each spot were detected as mentioned in Section 2.4. Serial concentrations of Phe–acetone and Fla–acetone also with fixed concentration of the other two PAHs were also prepared and determined with the same approach, respectively. Results (Table 1) revealed that the amount of Flu, Phe and Fla showed a good linear relationship with their relative fluorescence intensities. These experimental results indicated that the fiber-optic fluorimetry method can be used as an *in situ* analysis method for simultaneous quantify Flu, Phe and Fla adsorbed on living *Kc* leaves during their photolysis processes.

3.3. Interference experiment

PAHs often exist as a mixture with a great variation of their concentrations in the natural environment, for the accurate determination of them, it is very necessary to make sure that the determination of the three PAHs did not influence each other. Hence the interference experiments were performed. The experimental methods and procedures were as same as that mentioned in references [32,33]. Results were listed in Table 2. As can be seen from Table 2, when the concentration of Flu was fixed at the middle level (400 ng/spot) in its linear dynamics range, with increasing Phe and Fla concentrations, there was no significant influence on the fluorescence signal of Flu caused by the two co-existing components. The RSD for determination of the Flu was 1.55%. Similar conclusions can be drawn from the results for Phe and Fla, and their RSD values were 1.98% and 1.41%, respectively. From these results, it can be seen that the precision of fiber-optic fluorimetry method was acceptable.

3.4. Recovery experiment

To verify the accuracy of the established method, recovery experiments were performed. For the recovery experiments of Flu, the three PAHs in mixtures in the acetone solution whose concentrations were 200, 250 and 100 mg/L for Flu, Phe and Fla were prepared at first, and 2 μL of the solution were introduced onto the leaf surfaces of *Kc* seedlings to make the concentration of Flu, Phe and Fla 400, 500 and 200 ng/spot for each spot. Then series concentrations of Flu were added to each of the same position. Their relative fluorescence intensities were detected after the volatilization of acetone. The concentrations of the added Flu were calculated according to its corresponding calibration equation. The recovery experiments of Phe and Fla were performed, and their recoveries were calculated by the same method. Results were shown in Table 3. From Table 3, it can be seen that the recoveries for Flu, Phe and Fla adsorbed on the leaf surfaces of *Kc* seedlings were among 83.0–91.2%, 78.5–88.5% and 91.5–107.3%, respectively ($n = 9$). This illustrated that the accuracy of the method was satisfactory.

3.5. Photolysis of the three PAHs adsorbed on the leaves of Kc seedlings

3.5.1. Results of the photodegradation experiment

The photolysis experimental results of the three PAHs adsorbed on the leaf surfaces of *Kc* seedlings were shown in Figs. 4 and 5(A, B and C) and Table 4. As shown in Fig. 4, plots of natural logarithms of the Flu, Phe and Fla concentrations variation versus time essentially yielded straight lines. Therefore the photolysis processes of Flu, Phe and Fla individual and in mixtures followed first order kinetics that was similar to the results presented in previous studies [26–28]. The disappearance rate constants of PAHs in the control experiments (k_C) were calculated, which were listed in Table 4. The fluorescence spectra of the three PAHs in mixtures adsorbed on the leaf surfaces of *Kc* seedlings after a different certain time interval during the photolysis period were presented in Fig. 5. The decreases in the relative fluorescence intensities of the three PAHs were observed, while the shapes of the three PAHs fluorescence spectra were no significant changes during the photolysis period. The total disappearance rate constants (k_T) and k_C were determined from the slope of the line fitted by least squares regression analysis [25–27], $\ln(C_0/C_t) = k_t$, where C_t is the concentration of the three PAHs at time 't' during the illumination exposure, and C_0 is the initial concentration of the three PAHs. Thus, the photolysis rate constant (k_P) can be calculated by subtracting k_T from k_C. R_T^2 values listed in Table 4 were taken as

Table 6
Photodegradation rates determined in this study, normalized to average conditions and related to other studies.

PAHs	Photolysis half-lives of PAHs (h)				
	Measured in average value	Normalized data[a]	Literature data for spruce, pine needles		
			Ref. [26]	Ref. [27]	Ref. [28]
Flu	0.29	12.8	41	96	65.4
Phe	0.91	40.3	75	158	51.0
Fla	0.75	33.2	26	151	25.6

[a] Normalized for temperature and light, to derive photodegradation half-lives on mangrove leaves under the average conditions in previous researches.

a measure of the goodness of fit for the total illumination period, and R_C^2 values were taken as a measure of the goodness of fit for the control experiments. The half-lives of the three PAHs individual and in mixtures adsorbed on the leaf surfaces of *Kc* seedlings were calculated by the formula of $t_{1/2} = \ln 2/k_P$ (Table 4).

As can be seen from Table 4, k_P values were far greater than k_C values. The photolysis rates of the three PAHs in mixtures were slowed down 13.8%, 19.0% and 15.5% compared to Flu, Phe and Fla individually adsorbed on leaf surfaces of *Kc* seedlings, respectively. In order to know exactly the proportion of the three PAHs disappeared by photolysis, volatilization and absorption, the final residue amount of the three PAHs at the end of the experiment were listed in Table 5. We found that the three PAHs individual or in mixtures adsorbed on the leaf surfaces of *Kc* seedlings could not be totally photolyzed. $\Delta C_P/C_0\%$ stands for the proportion of the disappearance of the three PAHs being photolyzed. $\Delta C_C/C_0\%$ stands for the total proportion of the disappearance of the three PAHs because of volatilization and absorption in the control experiment. It was obvious that the values of $\Delta C_P/C_0\%$ were about 3–11 times larger than those of $\Delta C_C/C_0\%$. From the data showed in Tables 4 and 5, we found that photolysis played an important role for their fate of the three PAHs individual and in mixtures adsorbed on the leaf surfaces of *Kc* seedlings under the laboratory conditions, and their disappearance percentage of absorption by *Kc* leaf and volatilization from *Kc* leaf surfaces were neglectable during the experimental period.

3.5.2. Possible mechanisms for the explanation of the photodegradation experiment obtained

The above photolysis experiments showed that the photolysis rates of the three PAHs in mixtures were decreased as compared to their photolysis rates as an individual component adsorbed on the leaf surfaces of *Kc* seedlings. This phenomenon indicated that PAHs in mixtures adsorbed on the leaf surfaces of *Kc* seedlings would exist longer compared to them individually in the environment. The reasons for the lower photolysis rates of the three PAHs in a mixture might be as follows: firstly, as some researches had shown, the excited state of horned or bended PAHs (such as Phe and Fla) in solutions could be inhibited by other PAHs (such as Flu). So it was difficult for them to be photolyzed [35–37], and this explanation might also be suitable for the PAHs adsorbed on leaf surfaces of *Kc* seedlings. Secondly, in a mixture of the three PAHs, attenuation and shading of the mercury lamp light can occur since there is an overlap in the absorbance spectra of the three PAHs (Fig. 6), hence, the ratios between the fractions of light absorbed by each of the three PAHs changes throughout the wavelength range. This would be a logical explanation for the antagonistic effect on the lower photolysis rates of the three PAHs in a mixture. Thirdly, since the photolysis processes of PAHs adsorbed on most plant surfaces mainly occur in plant–air interface, so the photolysis rates of PAHs have a close relationship to the concentration of reactive oxygen in the air such as $^1O_2$, $O_2^{\bullet -}$, $OH^\bullet$ and H_2O_2 [27,28]. And when the three PAHs were coexisted, the same amount of active oxygen would be shared by them that would certainly less than that of individual PAHs adsorbed on leaf surface of *Kc* seedlings. This would also decrease the photolysis rate of the three PAHs in mixtures.

3.5.3. Photolysis half-lives of the three PAHs

The half-lives of the three PAHs in our experiments were much smaller than those shown in some previous studies [26–28]. The possible reasons might as follows: most of the light emitted by mercury lamp was in UV-band with its highest energy at 365 nm (Fig. 6). As can be seen in Fig. 7, the main absorbance of the three PAHs were in the wavelength from 230 to 330 nm that belong to the UV-bond of the sunlight reaching the earth's surface. This indicated that the three PAHs adsorbed on mangrove leaves could be directly photolyzed under the irradiation of mercury lamp. The light intensity used in this study was higher because of the reasons mentioned in Section 2.5. Therefore, the photolysis rates of the three PAHs adsorbed on living *Kc* leaves were much faster than those showed in previous studies. It is commonly believed that the light intensity of sunlight reaching the earth's surface in a sunny day was about 10×10^4 lx, however, only about 4% of the total energy from sunlight reaching the earth's surface occurs in the UV band [27]. If it is assumed that the relationship between the photolysis rate and light intensity is linear [38], and the average sunlight intensity was 10×10^4 lx, then the data were 'normalized' to represent typical solar radiation values averaged for the conditions in the previous studies following the method showed in reference [38]. The photolysis rates measured here are about 22 times of those measured by the previous studies. Meanwhile, it was commonly believed that the photolysis rate of PAHs would be accelerated by the increase of temperature [39–41]. The experiments conducted here were performed at 25 °C, which may give about 2-fold increase over the photolysis rates reported in the previous reports [26–28]. Thus, after normalizing the data to the light intensity and temperatures, the photolysis rates of the three PAHs in this paper are about 44 times of those measured in the previous studies (see Table 6).

From Table 6, there were still some differences between our normalized data and that of in the previous studies. It has been reported that the photochemical behavior of PAHs is strongly dependent on the nature of the surface upon which the compound is sorbed [42]. Different vegetation were used in the published references, the microstructure, the composition and solar-selective absorption of the leaf-wax of the different leaves would also influence the results [26–28,43].

4. Conclusion

Using the established optical fiber fluorimetry, Flu, Phe and Fla in mixtures adsorbed on the leaf surface of living *Kc* seedlings were *in situ* simultaneously determined. Meanwhile, the photolysis processes of the three PAHs adsorbed on the leaf surfaces of *Kc* seedlings were also studied for the first time. The results showed that the established method is simple, rapid, easy operating, accurate and good enough to be used as an *in situ* quantitative determination of the three PAHs individual and in mixtures adsorbed on the leaf surfaces of living *Kc* seedlings. The photolysis rates of

the three PAHs individual and in mixtures were in the same order of Flu > Fla > Phe, and when different kinds of PAHs were coexisting in the environment, the photolysis rates of them were slowed down. The results also indicated that photolysis of the Flu, Phe and Fla individual and in mixtures adsorbed on mangrove leaves was the main disappearance pathway of PAHs adsorbed, with only a small amount of Flu, Phe and Fla disappearing by volatilization and absorbing by leaves of *Kc* seedlings under laboratory conditions.

Compared to the methods showed in references [26–28,44], we can find that our method has the following advantages: firstly, the originally existing forms and the distribution of PAHs adsorbed on the leaf surfaces can be easily *in situ* determined, and it was impossible for the traditional method, such as GC/MS, GC and HPLC which need an extraction of sample before analysis. Secondly, no complex sample pretreatment is needed for this method, and it only needs less than 1 min for the determination of each sample. These merits make the established method not only environmental friendly and time-saving, but also easy to operate. Third, the established method has a great potential to be used in field as the experimental conditions were further optimized and the sensitive of the method were improved.

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