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In situ investigation of the depuration of fluoranthene adsorbed on the leaf surfaces of living mangrove seedlings

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ABSTRACT

A novel approach for the *in situ* determination of fluoranthene (Fla) that was adsorbed onto the leaf surfaces of *Avicennia marina* (*Am*), *Kandelia obovata* (*Ko*), *Bruguiera gymnorrhiza* (*Bg*), and *Aegiceras corniculatum* (*Ac*) was established using laser-induced nanosecond time-resolved fluorescence (LITRF). The detection limits for the *in situ* determination of the Fla adsorbed onto the *Am*, *Ko*, *Bg* and *Ac* leaf surfaces were 0.03, 0.14, 0.16 and 0.31 ng spot^{-1}, respectively. Using the LITRF method, the depuration of the adsorbed Fla from the leaf surfaces of the four selected mangrove species was investigated *in situ*. The results showed that the method was very simple and rapid to perform and it was applicable for a real-time *in situ* survey of the depuration processes of PAHs that were adsorbed onto the mangrove leaves. The depuration of Fla that was adsorbed onto the *Am*, *Ko*, *Bg* and *Ac* leaf surfaces followed fast and slow phases, both of which varied significantly between the mangrove species in terms of the elimination rate, the remaining Fla residues, and the effect of temperature on the remaining Fla residues on the leaf surfaces. Variations in leaf wax content and the leaf surface roughness among the four mangrove species were responsible for the differences in the rapid phase, while photolysis and penetration into the inner cuticle were dominant mechanisms for the slow depuration.

1. Introduction

Polycyclic aromatic hydrocarbons (PAHs), as a class of semi-volatile organic contaminants (SOCs) are ubiquitous and persistent in the environment, especially in the atmosphere [1,2], and some PAH compounds are known to be teratogenic, carcinogenic and mutagenic [3]. The aromatic structures and physicochemical properties of the PAH molecules facilitate their accumulation in organic matter and vegetation [4,5]. Accordingly, air–vegetation interactions play a significant role in influencing the air–surface exchange of PAHs and, hence, global cycling [6]. For example, various types of vegetation scavenge PAHs from the atmosphere, modify their deposition processes, and store and transfer them into other environmental compartments [7,8]. It is therefore vital that we understand the processes and mechanisms of air–vegetation exchange to improve the accuracy of human exposure and global environmental fate models [9–11].

It has been well documented in the literature that depuration or clearance experiment can be easier to perform and interpret than uptake experiment, and relevant studies have suggested that the depuration of SOCs from a whole leaf generally included a rapid phase and a slow one [12,13]. Accordingly, the whole leaf has been always modeled as a two-compartment system with respect to the depuration of PAHs that consists of a surface and a reservoir compartment. Moreover, the depuration processes are variable with different plant species and SOC compounds [14]. In summary, some good conclusions have been derived on the depuration processes of PAHs from a whole leaf of some vegetation types, however it cannot asserted whether PAHs in other plant species, especially from some special ecological environments, have the same or similar depuration behaviors. In addition, plant cuticles, as a hydrophobic lipid structure, have been reported to be high efficiency natural sorbents for PAHs and seemed to be a good reservoir for organic contaminant storage [15], and the fate of PAHs will be influenced by their location in/on the plant. For example, the PAHs adsorbed onto the relatively impermeable cuticular surface may undergo photolytic degradation [16], partition back to the air, or diffuse further into the cuticle and ultimately other lipid compartments deeper in the leaf [17]. However, few works have been conducted to *in situ* investigate the processes and mechanisms for the depuration of PAHs from the leaf wax surface of living plant.

In the relevant published works, the analytical methods that have been used have focused on GC, GC/MS and HPLC. Although these techniques are characterized by high precision and accuracy,

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they typically require chemical extraction steps that destroy the original forms of the target compounds. Thus, the results derived using these methods cannot accurately reflect PAH exchange processes between the air and individual compartments of the plant leaf. Because PAH compounds have a relatively high fluorescence quantum yield, we developed a series of *in situ* methods, including solid-surface fluorimetry (SSF) [18], synchronous solid-surface fluorimetry (S-SSF) [19] and fiber-optic fluorimetric technique [20,21] to determine the *in situ* adsorption of PAHs onto mangrove leaf surfaces. However, limited by the sensitivity and non-portable instrument (a Cary Eclipse fluorescence spectrophotometer, Varian, USA), it still remains difficult to conduct field measurements of PAHs using these *in situ* methods. Portable laser-induced nanosecond time-resolved fluorescence (LITRF) system is fairly accepted as a fast and reliable method for the field monitoring of PAHs in various environmental media [22]. In addition, a novel method for the *in situ* monitoring of phenanthrene (Phe) adsorbed onto mangrove leaf surfaces has been established under laboratory conditions using LITRF in the lab [23]. However, the question of whether LITRF can be used for real-time *in situ* investigations into the depuration processes of PAHs adsorbed onto the leaf surfaces of living plant has not yet been approached.

Mangrove ecosystems are unique transitional coastal ecosystems between marine and terrestrial environments that are generally confined to tropical and subtropical regions [24], and research on mangrove pollution ecology in recent years has increased considerably [25]. Mangrove leaves possess large areas and thick lipid cuticles to be able to adapt to their special ecological habitats. Theoretically, mangrove leaf surfaces have a great potential to adsorb atmospheric PAHs. As reported in our previous studies, the capacity for trapping PAHs was different between mangrove species [18], and under the irradiation of high pressure Hg lamp, photolysis was found to be the main transformation pathway for the PAHs adsorbed on the mangrove leaf surfaces, while disappearance of the adsorbed PAHs as a result of volatilization and adsorption could be negligible [20,21]. However, the results derived cannot accurately explain the processes and mechanisms for the depuration of adsorbed PAHs under conditions of a simulating the ecological system and real environment. In this work, a novel established LITRF method was used for *in situ* investigation of the depuration of Fla adsorbed onto the leaf surfaces of living *Avicennia marina* (*Am*), *Kandelia obovata* (*Ko*), *Bruguiera gymnorrhiza* (*Bg*), and *Aegiceras corniculatum* (*Ac*) seedlings.

2. Materials and methods

2.1. Apparatus and reagents

All the spectra were obtained using a LITRF, which was purchased from Laser Laboratorium Gottingen GmbH, Germany. The system is composed of four parts: a class 4 neodymium-doped yttrium aluminum garnet (ND:YAG) diode laser that emits UV pulses (266 nm) of up to 40 μJ/pulse, with 1.0 ns pulse duration and a max pulse repetition rate of 50 Hz, which allows for the detection of steady-state and transient fluorescence spectra simultaneously; an optical probe with a 15 m length optical fiber; an optical multi-channel (675 channels) analyzer with a time resolution of 6 ns, including a spectrograph, a gateable image intensifier and a charge-coupled device (CCD) camera; and a control and data acquisition unit (single board computer, SBC) [22].

A 10 μL flat head micro-injections (Shanghai Medical Laser Instrument Plant, China) was used to introduce Fla solution onto mangrove leaf surfaces. A large circle end of 5 mL pipette was used to produce the determination area on the leaf surface.

The Fla stock solution was prepared according to the previously described method [20].

2.2. Sample collection

Mature viviparous propagules of *Am*, *Ko*, *Bg* and *Ac* were collected from the 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) and then quickly transported to the laboratory for cultivation in a sand bed for 12 months. Then, the mangrove seedlings of approximately the same height and maturity were selected for the following experiments.

2.3. Pretreatment of mangrove seedlings for the experiment

Nine living seedlings of each mangrove species that were similar in height were selected. To remove the surface silt, all of the selected leaves were carefully rinsed with tap water and Milli-Q water three times. After air drying, a 'spot' unit, which was used as the determination location, was produced according to the method published in the laboratory [21]. Then, working solutions of Fla in acetone were introduced onto these 'spots' using a 10 μL flat head micro-injector under the experimental conditions in the laboratory.

2.4. Determination of the Fla adsorbed onto the mangrove leaf surfaces

The quantities of Fla adsorbed onto the leaf surfaces of four selected mangrove species were directly determined using the LITRF method. The LITRF parameters settings are listed in Table 1. The marked 'Fla spots' on the mangrove leaves were placed under the optical fiber probe of the LITRF system, which conducted the leaf surface lightly, and the leaves were kept smooth during the experiment. The detection areas were selected at the front, middle and rear parts of the leaves, as shown in the literature [18]. The average values of 9 measurements from each leaf were used as the final results, and the relative deviations ($n=9$) were also calculated.

2.5. Depuration experiment

After the leaves were contaminated by Fla, each mangrove species was then divided into three sets. One set, including three seedlings, was transferred into a greenhouse where the temperature was 22 ± 3 °C, while the other two sets were transferred into two separate plant growth chambers in which the temperatures were set at 10 ± 2 °C and 30 ± 2 °C. The *in situ* determination of the Fla adsorbed on the mangrove leaf surfaces was conducted at 0, 1, 2, 4, 6, 8, 12, 18, 24, 36, 48, 72, 120, and 180 h for the set in the greenhouse to follow the clearance kinetics and conducted only twice (at 0 and 180 h) for the sets in the plant growth chambers to

Table 1
LITRF parameters for the measurement of the Fla adsorbed onto the mangrove leaf surfaces.

Parameters	Gain value settings	Determining parameter settings
Timing: start (ns)	75	75
Timing: width (ns)	100	5
Timing: shift (ns)	1	1
Lambda excitation (nm)	266	266
Laser energy (μJ)	40	40
Time slices (ns)	1	40
Channels	674	674
Cooler temperature (°C)	−8	−8

investigate the effects of temperature on the final residues of Fla on the mangrove leaf surfaces.

2.6. Statistical analysis

In this work, the determination of Fla adsorbed onto the mangrove leaf surfaces and the depuration experiments were all performed 9 times, and the mean values were used as the final results. Statistical analyses for the variations in the fluorescence intensities obtained by the LITRF method were performed using the statistics package for social science (SPSS) 13.0 for Windows. The significant differences in the results were determined using a one-sample *t*-test at the 95% confidence level.

3. Results and discussion

3.1. Fluorescence emission spectra of Fla adsorbed onto mangrove leaf surfaces

The optimal detection wavelengths of the Fla that was adsorbed on each type of mangrove leaf surface were first determined. As mentioned in Section 2.1, The LITRF has a single excitation wavelength (λ_{ex}) located at 266 nm. The emission wavelengths (λ_{em}) of the Fla adsorbed on the four types of mangrove leaves were determined as follows: 465 nm for *Am*, 466 nm for *Ko*, 467 nm for *Bg*, and 466 nm for *Ac*. To ensure that the autofluorescence of each mangrove leaf would not interfere with the *in situ* determination of Fla, the fluorescence spectra of the uncontaminated leaves while using acetone as a blank were also obtained using the established method. The results are shown in Fig. 1. Fig. 1 shows that neither the autofluorescence of the uncontaminated *Ko* leaves nor the acetone as a blank should interfere with the measurements of the Fla adsorbed on the leaf surfaces. Similar results were also observed for the other three types of mangroves (results are not shown here). It was evident that the established LITRF method was acceptable for use as an *in situ* approach for the determination of the amount of Fla adsorbed onto the surfaces of the mangrove leaves.

3.2. Analytical merits of the established method

It has previously been reported that the amount of PAHs adsorbed onto the vegetation leaves are very low in the actual environment [26]. Therefore, it is of great necessity to develop a novel method with high sensitivity and accuracy. Importantly, the accurate quantification of the amount of Fla adsorbed onto the surfaces of the vegetation leaves was a prerequisite for the following depuration experiment. According to the method reported in the laboratory [21], different concentrations of Fla in acetone solutions were prepared and introduced uniformly onto the spots of the tested mangrove leaf surfaces, and the relative fluorescence intensities from each spot were detected using the LITRF method. The experimental results are summarized in Table 2. The results in Table 2 show that the concentration of Fla that was adsorbed onto the leaf surfaces of the four mangrove species showed a good linear relationship with their relative fluorescence intensities within a certain range. They also show that some differences existed between the linear dynamic ranges and the detection limits among the four tested mangrove species. This could be explained by the fact that the fluorescence intensities of the same amounts of fluorescent materials were different between the different surface properties of the adsorbent matrices [27]. For example, the leaf surface roughness was considered to be an important factor in influencing the detection of the relative solid surface fluorescence intensities of PAHs adsorbed onto the mangrove leaves [18].

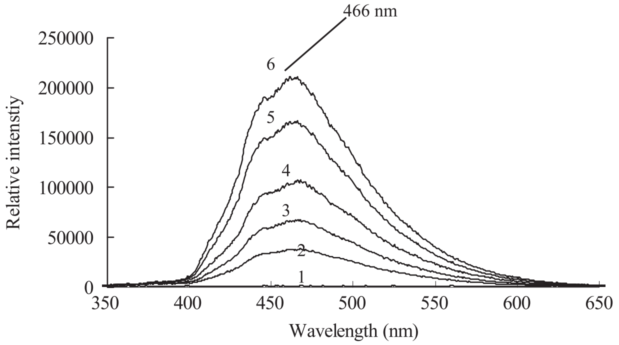

Fig. 1. Emission spectra of the Fla adsorbed onto *Ko* leaf surface (λ_{ex}=266 nm). Concentrations of the Fla adsorbed: 1—blank; 2—100; 3—200; 4—400; 5—600; 6—800 (ng spot^{-1}).

As reported in our previous study, an analytical approach for the determination of Fla that was adsorbed onto *Excoecarla agallocha* (*Ea*), *Ko*, and *Ac* leaves was also established using fiber optical fluorimetry (Varian, USA), with linear dynamic ranges of 2.5–680, 1.0–620 and 10–710 ng spot^{-1}, respectively, in addition to the detection limits ranging from 0.80 to 3.01 ng spot^{-1} [20]. Therefore, compared with the established the fiber optic fluorimetry method, the sensitivity of the LITRF method was increased by a factor of 10–30, and its linear dynamic ranges were also improved. The results marked a significant step toward realizing the field detection of PAHs in vegetation.

3.3. Recovery experiment

To confirm the feasibility and accuracy of the LITRF method for the *in situ* determination of the amount of Fla adsorbed onto the leaf surfaces of the four mangrove species, a recovery experiment was performed using the method reported in the laboratory [19–21], and the experimental results are shown in Table 3. These results showed that the recoveries for the Fla adsorbed onto the *Am*, *Ko*, *Bg* and *Ac* leaf surfaces ranged from 91.8% to 110.0%, 98.3% to 114.6%, 96.2% to 111.2% and 95.6% to 104.0%, respectively. These results illustrated that the accuracy of the method could meet the requirements of the depuration experiment.

3.4. Depuration of Fla adsorbed onto the leaf surfaces of the selected mangrove species

It has previously been reported that the upper leaf surfaces have thicker epicuticular wax layers than the lower surfaces [20]. The cuticle and its surface epicuticular wax play a key role in the storage and re-release of lipophilic SOCs that reach the vegetation from the air [15]. Meanwhile, SOCs that have adsorbed onto the

Table 2
Analytical merits of the established method.

Mangrove	Calibration curve	Linear range (ng spot^{-1})	Correlation coefficient	Detection limit (ng spot^{-1})[a]
Am	y[b]$=297.6x$[c]$+7800$	0.1–800	0.9952	0.03
Ko	$y=269.5x+5725$	0.3–1080	0.9973	0.11
Bg	$y=239.7x+1068$	0.7–1260	0.9979	0.16
Ac	$y=264.9x+1883$	0.4–1130	0.9979	0.31

[a] Detection limit of the method, which was calculated by $3S_B/m$, where 'S_B' is the standard deviation of the blank, and 'm' is the slope of the calibration curve.

[b] y represents the fluorescence intensity of the Fla adsorbed onto the surfaces of the mangrove leaves.

[c] x represents the concentration of Fla added to the surfaces of the mangrove leaves.

Table 3
Results of recovery experiment for the Fla adsorbed onto the leaf surfaces of four selected mangrove seedlings.

Mangrove	Original ($ng\ spot^{-1}$)	Added ($ng\ spot^{-1}$)	Total ($ng\ spot^{-1}$)	Measured ($ng\ spot^{-1}$)	Recovery (%)
Am	50.0	50.0	105.0	55.0	110.0
	100.0	100.0	191.8	91.8	91.8
	200.0	200.0	396.5	196.5	98.3
Ko	100.0	100.0	198.3	98.3	98.3
	200.0	100.0	314.6	114.6	114.6
	400.0	200.0	612.3	212.3	106.2
Bg	100.0	100.0	196.3	96.3	96.3
	200.0	100.0	311.2	111.2	111.2
	300.0	200.0	492.4	192.4	96.2
Ac	100.0	100.0	195.6	95.6	95.6
	200.0	100.0	297.8	97.8	97.8
	400.0	200.0	607.9	207.9	104.0

upper leaf surfaces can diffuse further into the cuticle and ultimately other lipid compartments that are deeper within the leaf [28], they may re-volatilize into the air if environmental conditions change (e.g., ambient concentrations drop or temperature increases), or they may undergo photolysis. To elucidate the main fate of the Fla that was adsorbed onto the upper leaf surfaces, the depuration processes of the Fla from the four selected mangrove species were investigated *in situ* using the LITRF method. The experimental results are shown in Fig. 2. It was clear from the results that the depuration of the Fla adsorbed onto the mangrove leaf surfaces followed two-phase clearance kinetics. A rapid depuration occurred during the initial 8 h, while a slower depuration followed and lasted approximately 172 h. These observations were consistent with those reported for other SOCs (e.g., PCBs, OCPs) in previous studies [13,29]. In the previous studies, the kinetic depuration processes were modeled using a first-order clearance model, and the slopes of the plots represented the elimination rate constant.

$$\ln C_L = \ln C_{L(0)} - kt$$

where $C_{L(0)}$ represents the initial concentration of the compound accumulated in leaf, and k is the elimination rate constant.

As mentioned in Section 3.2, the concentrations of Fla that was adsorbed onto the leaf surfaces of the four mangrove species exhibited good linear relationships with their relative fluorescence intensities. Therefore, to display the differences observed during the slow depuration more clearly, ln I_v was plotted against time for the slow depuration process.

$$\ln I_{L(t)} = \ln I_{L(0)} - kt$$

where $I_{L(t)}$ is the relative fluorescence intensity of the Fla that was adsorbed onto the mangrove leaf surfaces at time t, and $I_{L(0)}$ is the relative fluorescence intensity of Fla that was adsorbed onto the mangrove leaf surfaces at the start of depuration. It can be seen from Fig. 3 that ln $I_{L(t)}$ of the Fla adsorbed onto the mangrove leaf surfaces versus time essentially yielded straight lines during both the fast and slow depuration processes. Therefore, we concluded that the depuration of the Fla that was adsorbed onto the mangrove leaf surfaces followed first-order kinetics. Table 4 displays the kinetic parameters for the depuration of Fla adsorbed onto the leaf surfaces of the four selected mangrove seedlings. It can be seen that, within the same mangrove species, the elimination rate constants of Fla varied little with the different initial concentrations of the Fla sorbed on the mangrove leaf surfaces whether for rapid and slow phase ($p > 0.05$), leading to the conclusion that the depuration process was independent of the initial

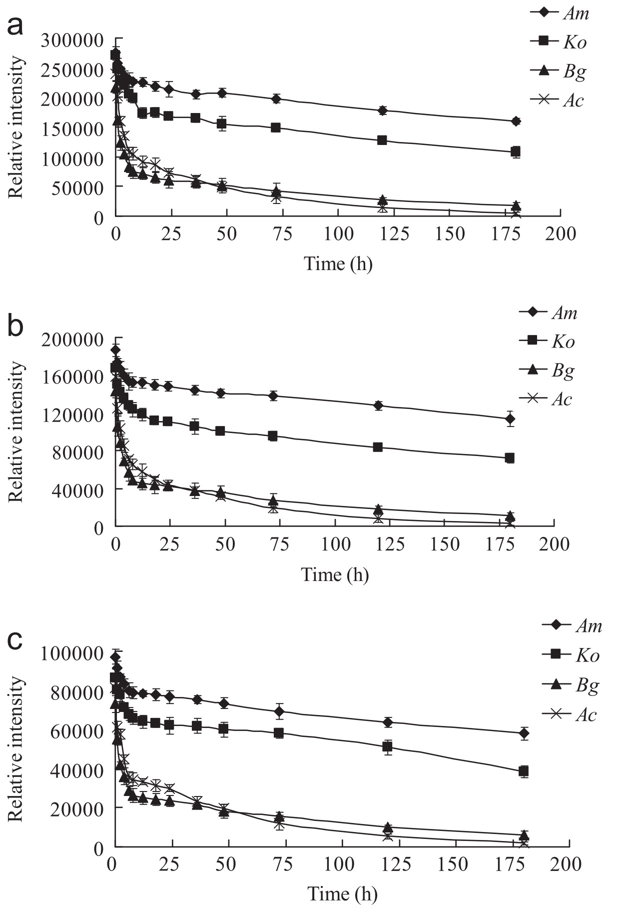

Fig. 2. Depuration kinetics of the Fla with different initial concentrations on the leaf surfaces of four selected mangrove species. Initial concentrations of Fla: (a) 300; (b) 600; (c) 900 ($ng\ spot^{-1}$). The error bars represent the standard deviations of 9 measurements.

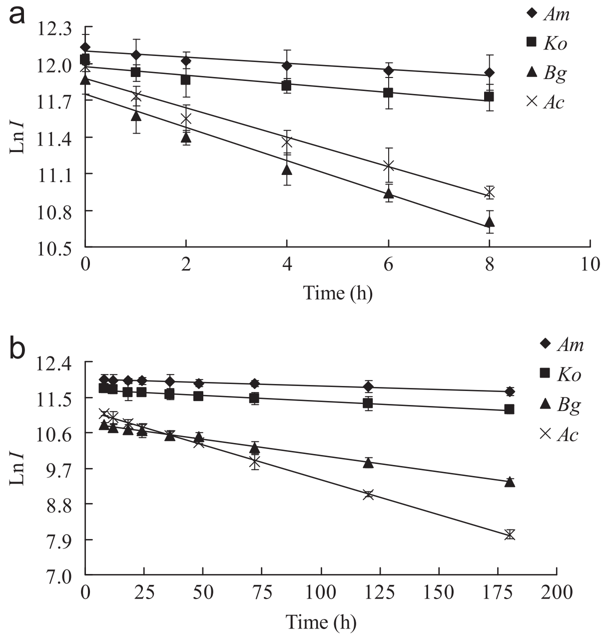

Fig. 3. Plot of ln *I* (relative intensity) vs. time during the fast (a) and slow (b) depuration processes. Initial concentrations of the Fla adsorbed onto the leaf surfaces of the four selected mangrove species: 600 $ng\ spot^{-1}$. The error bars represent the standard deviations of 9 measurements.

Table 4
The kinetic parameters for the depuration of Fla adsorbed onto the mangrove leaf surfaces ($n=9$).

Mangrove	Fla (ng spot^{-1})	k_1 (h^{-1})	R_1^2	k_2 (h^{-1})	R_2^2	k
Am	300	0.0307	0.9279	0.0018	0.9968	0.0023
	600	0.0298	0.9162	0.0017	0.9942	0.0021
	900	0.0284	0.9108	0.0020	0.9841	0.0025
Ko	300	0.0398	0.9713	0.0029	0.9571	0.0037
	600	0.0411	0.9025	0.0030	0.9766	0.0039
	900	0.0417	0.9085	0.0031	0.9596	0.0042
Bg	300	0.1464	0.9311	0.0086	0.9979	0.0109
	600	0.1474	0.9537	0.0083	0.9968	0.0109
	900	0.1483	0.9268	0.0084	0.9972	0.0111
Ac	400	0.1286	0.9671	0.0173	0.9982	0.0187
	800	0.1282	0.9553	0.0176	0.9986	0.0198
	1200	0.1295	0.9704	0.0177	0.9934	0.0196

k_1—represents the depuration rate constant during the rapid phase; k_2—represents the depuration rate constant during the slow phase; k—represents the overall depuration rate constant.

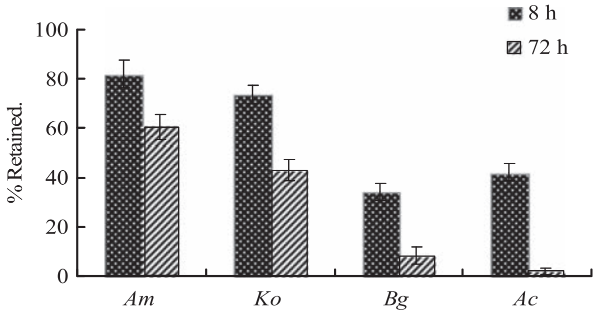

Fig. 4. Percentage of the Fla retained on the leaf surfaces of four selected mangrove species after 8 and 180 h depuration. Initial concentrations of Fla: 600 ng spot^{-1}. The error bars represent the standard deviations of 9 measurements.

concentrations of Fla. In contrast, the experimental results also showed that significant differences existed in both the rapid and slow kinetic depuration phases between the mangrove species. Therefore, the depuration processes of adsorbed Fla with the initial concentrations of 600 ng spot^{-1} were taken as an example to illustrate the differences between the selected mangrove species.

3.4.1. *Rapid depuration phase*

As seen from Fig. 2, the depuration of the Fla from the four selected mangrove species was the fastest during the initial 8 h, which resulted in a substantial loss of Fla burden that the mangrove leaf could have adsorbed. The initial rapid loss of the Fla burden most likely represented a rapid exchange between a surface compartments and the air. As previously reported, the fast-exchanging surface compartments have response times of hours to changes in air concentrations [29]. If the process responsible for this loss was simply the desorption of the Fla that had adsorbed onto the leaf surface and that had not penetrated the cuticle, then the clearance rates or fraction lost for the rapid clearing process should vary between different mangrove species, as the capacity for trapping PAHs was reported to be different between mangrove species [18]. The possible mechanism could be confirmed by the results shown in Table 4 and Fig. 4. Table 4 shows that the clearance rates during the fast phase were variable between the selected mangrove species, with the values decreasing in the order of *Bg* > *Ac* > *Ko* > *Am*. Meanwhile, Fig. 4 also shows that significant differences existed between *Am*, *Ko*, *Bg* and *Ac* in terms of the percentage losses of the Fla burden that had accumulated on the leaf surfaces after the initial 8 h depuration ($p < 0.05$). Hiatt [30] suggested that the capability of a leaf for trapping a compound was closely associated with the properties of the compound and the vegetation. To further illustrate the results shown in Fig. 4, the leaf wax contents of the four species were determined using the method published previously in the lab [31], and the values for the *Am*, *Ko*, *Bg*, and *Ac* leaves (mean ± SD) were 9.5 ± 4.2, 6.7 ± 3.1, 5.7 ± 3.0 and 5.2 ± 2.7 mg g^{-1}, respectively, which were consistent with the previous results reported for similar mangrove species [18]. *Bg* and *Ac*, with their low leaf wax content, could not trap Fla tightly, while *Am* and *Ko*, containing higher leaf wax content, were able to retain Fla more effectively (see Fig. 4). These results corroborated observations from Wang et al. [18] that, under the same exposure conditions, greater leaf wax content was associated with the increased capabilities of mangrove leaves to trap PAHs. However, no correlation existed between the Fla residues and the leaf wax content for the species containing moderate leaf wax content, and even a negative correlation existed for *Bg* and *Ac*. This might have been associated with other vegetation parameters that are often considered to affect PAH concentrations in leaves, such as specific leaf area, lipid content and leaf surface roughness [32]. Furthermore, it has previously been reported that the differences in the forms of leaf wax between mangrove species were shown to influence the adsorption of anthracene (An) onto leaf surfaces [18]. *Am* contains salt glands on its leaf surfaces, making it much coarser than the other mangrove leaves. Therefore, this observation implied that rough (for example, *Ac*) leaves retained greater amounts of Fla residues than flat and smooths (for example, *Bg*) leaves. This result was consistent with those in other previous studies [7,33].

3.4.2. *Slow depuration phase*

It can be seen from Table 4 that, the slow depuration trends exhibited significant differences ($p < 0.05$) between the four selected mangrove species in terms of the elimination rate constants of Fla, which ranged from 0.0017 to 0.0020, 0.0029 to 0.0031, 0.0083 to 0.0086 and 0.0173 to 0.0177 h^{-1} for *Am*, *Ko*, *Bg*, and *Ac*, respectively.

It was necessary to consider whether the slow depuration of the Fla from the leaf surfaces was largely ascribed to volatilization or due to other processes, notably photolysis or penetration into the inner cuticle. If volatilization were significant, the elimination rates of Fla would be expected to be nearly identical among the four selected mangrove species, whereas the elimination rates varied between the mangrove species. This observation suggested that volatilization was not the dominant mechanism of the slow depuration. Another possible explanation for the slow clearing process was the photolytic degradation of the SOCs adsorbed onto the leaf surfaces. Experimental results have shown that the photodegradation of PAHs does occur in plant cuticular wax [34]. Additionally, the photodegradation of Fla that was adsorbed onto *Ko*, *Excoecarla agallocha* (*Ea*), and *Ac* leaf surfaces have been investigated *in situ*, and great variations were observed in the photolysis rates, with the rates decreased in the order of *Ac* > *Ea* > *Ko* [20], which could partially explain the more rapid loss of Fla from the *Ac* leaf surfaces during the slow depuration phase. Another possible explanation for the slow depuration was diffusion into the cuticle. For example, Wild et al. [17] reported that An could move through the epicuticular wax and plant cuticle and reach the cytoplasm of the epidermal cells of living maize leaves. Similarly, we also observed that both Phe and Fla that were adsorbed onto *Am* leaf surfaces could diffuse into the inner leaf tissues and transfer within the plant tissues (unpublished data). The diffusion rate was reported to be regulated by the factors such as composition and the permeability of the cuticular skin [35]. As can be seen from Fig. 4, the final Fla residues on the leaf surfaces at the end of the depuration experiment was found to exhibit a significant positive correlation with the leaf wax content among the four mangrove species. The cuticular skin on the leaf surface has been shown to be a very impermeable structure for the diffusion of organic chemicals due to the presence of a crystalline wax layer. Generally, less leaf wax content causes greater amounts of chemicals to diffuse into the cuticle, while greater leaf wax content results in more amounts of chemicals that are retained on the leaf surface [36]. In summary, photolysis and diffusion into the cuticle were considered as important mechanisms of the slow depuration process.

3.4.3. *Fla residues on mangrove leaf surfaces after 180 h depuration*

Also from Fig. 4, it can be seen that the loss of Fla was almost complete from the *Bg* and *Ac* leaf surfaces, with only 1.9% and 8.2% of the Fla remaining after 180 h depuration, respectively. In contrast, the amount of remaining Fla residues on the *Am* and *Ko* leaf surfaces was 39.9% and 61.2%, respectively. In addition, it is very interesting to note that the concentration of Fla that was adsorbed onto the *Bg* leaf surfaces was lower than the amount that was adsorbed onto the *Ac* leaf surfaces after the onset of the 8 h depuration, but the concentration of Fla that remained on the *Bg* leaf surfaces was higher than that on the *Ac* leaf surfaces at the end of the 180 h depuration (see Fig. 4). These results suggested the following: (i) Fla was retained effectively by both the *Am* and *Ko* leaf surfaces, while Fla was not trapped tightly by either the *Bg* or *Ac* leaf surfaces; (ii) the interactions between Fla and the mangrove species varied widely; and (iii) the remaining Fla residue was independent of its initial concentration, but it was closely associated with the type of mangrove species.

3.4.4. *Effects of temperature on the remaining Fla residue on the mangrove leaf surfaces after 180 h*

Temperatures in the environment vary both spatially and temporally, and they can significantly affect the air/plant exchange processes and the fates of the SOCs [13]. Different geographical locations experience different ambient temperatures, and these ambient temperature can vary seasonally. These differences in temperature affect the air/plant partitioning of SOCs and exchange rates of SOCs. Previous studies have implied that the concentrations of SOCs in vegetation exhibit seasonal patterns [37]. For example, Simonich and Hites [9] reported that PAHs were susceptible to accumulation in vegetation during the cold weather. Similarly, Smith et al. [4] found that selected PAHs, except for acenaphthene, exhibited strong seasonal fluctuations, and increased concentrations were observed in pine needles during the winter periods. Thus, in this work, the effects of temperature on the exchange of Fla between the air and the leaf surfaces of the four selected mangrove species were also investigated *in situ*. Fig. 5 displays the percentage of the Fla burden that remained on the leaf surfaces after 180 h depuration. In general, lower temperatures favored higher concentrations of Fla that were retained on the leaf surfaces, while higher temperatures led to more Fla partitioning into the atmosphere. However, it should be noted that the degree to which temperature affected the Fla residue varied between the different mangrove species. For example, the differences of remaining Fla residues on the *Ac* leaf surfaces caused by temperature were slight, ranging from 4.5% to 0.8%. This could have been attributed to their lower leaf wax content that restricted the adsorption and re-release of Fla. In contrast, significant differences were observed for both the *Am* and *Ko* species, which ranged from 72.4% to 37.0% and 25.3% to 52.2%, respectively. The dissimilarity observed between the selected mangrove species was most likely related to the different interactions between the compound and the unique vegetation species.

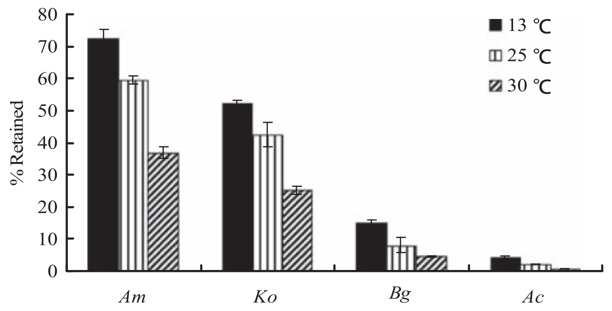

Fig. 5. Percentage of Fla that was retained on the leaf surfaces of four selected mangrove species after 180 h depuration under different temperatures. The error bars represent the standard deviations of 9 measurements.

4. Conclusions

The experimental results presented above showed that the newly established LITRF method not only enabled the *in situ* determination of Fla that was adsorbed onto the leaf surfaces of the four selected mangrove species, but also it was used to conveniently investigate the real-time *in situ* exchange of Fla between the air and the leaf surfaces of the four selected mangrove species. Considerable differences in the depuration process existed between the selected mangrove species in terms of the elimination rate and remaining amount of Fla. The variability was most likely due to the photolysis of the target compound, in addition to the composition and the permeability of the cuticular skin. Moreover, the fact that the remaining amounts of Fla residues on the leaf surfaces of the four mangrove species were influenced to different extents by temperature suggested the existence of variabilities between the compound and the vegetation species in the leaf-to-air transfer processes. Our study provided insight into the mechanism of the air–vegetation exchange and the behaviors of PAHs in the four types of selected mangrove species.

On the base of remaining significant advantages of those established *in situ* methods (e.g., SSF, the optical fiber fluorimetry), the accuracy, sensitivity and detection range of the newly developed method got a significant improvement, plus the LITRF system is characterized by high integration and portability, making the method possess great potential to be developed into a field monitoring technique for the *in situ* determination of PAHs that have been adsorbed onto the leaf surfaces of living plant. However, many more studies must be conducted in the near future. For example, PAHs in the environment typically exist in mixtures or multi-components, thus LITRF could be further explored to develop a new analytical method for the simultaneous determination of multi-component PAHs based on its nanosecond time-resolved ability.

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