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原位在线研究典型多环芳烃在红树植物
中的环境行为

In Situ Study on the Environmental Behaviors of Typical
PAHs in Mangrove Plants

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申请厦门大学博士学位论文

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在红树植物中的环境行为



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摘要

红树林是生长在热带、亚热带陆海交汇的海湾河口区潮间带、受周期性潮水浸淹的潮滩湿地木本生物群落。近年来随着人口的增长,流域工农业、沿岸城市开发及港口驳岸海运的发展,大量污染物直接或间接注入而汇集于河口海湾区,日益增加了红树林的环境压力。红树林生态系统因生产力高、富含有机质以及强还原性环境条件等特性,使之成为吸收和积累多环芳烃(Polycyclic Aromatic Hydrocarbons, PAHs)的重要场所,但目前有关红树林湿地中 PAHs 的研究大多限于分析湿地沉积物中 PAHs 的种类、各种组分的含量与分布比例、湿地内 PAHs 的来源研究以及林地内微生物对 PAHs 的降解作用等,而作为湿地主体核心的红树植物对环境中 PAHs 的直接吸收研究很少涉及,亦未对相关机理加以阐释。

本文首先利用固体表面荧光(Solid Surface Fluorimetry, SSF)分析法结合荧光显微(Fluorescence Microscopy, FM)和双光子激光共焦扫描显微(Two Photon Laser Confocal Scanning Microscopy, TPLCSM)技术,建立了红树林生态系统中典型 PAHs 环境行为原位研究的系统方法,进而利用所建方法初步开展了原位研究典型 PAHs 在红树植物中环境行为等工作,主要研究内容和结果包括以下几个方面:

(1) 利用荧光分光光度计配合自制的固体样品架建立了直接测定吸附于白骨壤(*Avicennia marina*, *Am*)、木榄(*Bruguiera gymnorrhiza*, *Bg*)、秋茄(*Kandelia candel*, *Kc*)和红海榄(*Rhizophora stylosa*, *Rs*)叶片上 An 的 SSF 新方法。实验结果表明:(a) 在相同的暴露时间下,不同种红树叶片对 An 的吸附量不同,且在一定范围内,吸附于四种红树叶片上表面 An 的量与其 SSF 相对强度间有较好的线性关系,所建方法的回收率为 70.2%~110.8%,可满足实际测定的需要;(b) 在相同的暴露 An 浓度下,随 An 暴露时间的增加,四种红树叶片上表面富集 An 后测得的 SSF 相对强度都呈现先增大后减小的趋势,且测得吸附于四种红树叶片上表面 An 的 SSF 相对强度最高处的顺序为: $Am > Kc > Rs > Bg$; (c) 在相同的实验暴露条件下,同种红树叶片上表面吸附 An 后测得的 SSF 相对强度都明显强于下表面,但下表面对 An 的吸附作用亦很重要。实验还发现,吸附在四种红树叶

片上表面 An 的 SSF 相对强度随吸附部位的不同都表现出较大差异,即可能存在于叶片表面的 An 并不是“均匀”地穿过表皮蜡质层进入到叶片内部,而是可能存在“优先”的进入位置,但具体原因尚不清楚。

(2) 在上述工作研究基础上,首先,利用荧光分光光度计配合光纤附件建立了直接测定吸附于 *Kc*、桐花树 (*Aegiceras corniculatum Ac*) 两种红树叶片上表面单组分及其混合组分 An、芘 (Py) 的光纤同步 SSF 新方法。实验结果表明,在一定范围内,吸附于两种红树叶片上表面单组分及其混合组分 An、Py 的量分别与其同步 SSF 相对强度间有较好的线性关系,且所建方法的回收率为 82.9%~116.5%,可满足实际测定的需要;其次,采用所建立的光纤同步 SSF 法原位研究了存在于活体 *Kc* 叶片上表面 An 的光谱行为,同时辅助 GC-MS 手段验证了所建方法在实际测定过程中的可行性。实验结果表明,在一定的实验条件下,随暴露时间的延长,采用两种方法测得的存在于 *Kc* 叶片表面 An 的量的变化趋势基本一致;最后,采用所建立的光纤同步 SSF 法原位研究了 An、Py 及其混合组分在两种红树叶片上表面的光降解情况。实验结果表明,在实验条件下:(a) 吸附于两种红树叶片上表面的单组分 An、Py 的光降解过程均遵循一级反应动力学过程;在相同的实验条件下,吸附于红树叶片上表面 PAH 的含量越高,光降解的反应速率越慢,且吸附于同种红树叶片上相同物质质量的 Py 的降解速率远小于 An;(b) 吸附于两种红树叶片上表面的 An、Py 混合组的光降解过程均遵循一级反应动力学过程。而对于处于混合组分中相同量的 PAH,吸附于 *Kc* 叶片上表面 PAH 的光降解速率慢于吸附于 *Ac* 叶片上表面的 PAH。实验结果还表明,不管是 *Kc* 叶片还是 *Ac* 叶片,与吸附于叶片表面单组分 An 或 Py 的光降解作用相比,混合组分中 An 的光降解速率减慢,而 Py 的光降解速率加快;(c) 单组分及其混合组分 An、Py 在水溶液中的光降解过程均遵循一级反应动力学过程。对于相同量的单组分 An、Py,处于水溶液中的 PAH 的光降解速率都快于吸附于红树叶片表面的 PAH。在水溶液中,与单组分 An 或 Py 的光降解作用相比,混合组分中 An 的光降解速率减慢,而 Py 的光降解速率加快。

(3) 在人工模拟实验条件下,选取 An 为模型化合物并将其均匀地涂抹于活体 *Kc* 叶片的上表面,经过 96h 的暴露后,首先借助于所建的 SSF 方法,确定了

存在于 *Kc* 叶片上表面的目标污染物为 An, 其次借助于 FM 技术, 直接观察发现, 随着 An 暴露时间的增加, 存在于活体 *Kc* 叶片上表面 An 的量逐渐减少, 一定时间后, 存在于 *Kc* 叶片上表面的 An 会与大气中的 An 达到挥发和再沉降的“相对平衡状态”, 且随着 An 暴露时间的延长, 存在于 *Kc* 叶片上表面的 An 会通过上表面的蜡质层进入到第一、第二内皮层、上栅栏组织, 从大气再沉降至 *Kc* 叶片下表面的 An 会通过下表面的气孔进入到下皮层、下栅栏组织, 并最终都分别到达海绵组织。实验结果还表明, 经过 24h 的暴露后, 在 *Kc* 叶片的上、下栅栏组织和海绵组织内部都会观察到一些“淡蓝色”的光斑, 很可能是 An 的代谢产物。同时实验还发现, 在相同 An 暴露时间 (24h 后) 下, 存在于叶片“中央”An 的量会少于存在于叶片“边缘”An 的量, 即随着暴露 An 时间的延长, 存在于叶片“中央”An 的量减少的“更快”。此研究结果可能暗示了一条重要的信息, 即可能存在于红树叶片表面的 An 并不是“均匀”地穿过表皮蜡质层进入到叶片内部, 而是可能存在“优先”的进入位置。

(4) 在人工模拟实验条件下, 利用 TPLCSM 技术, 原位观察了典型 PAHs 从水培液迁移进入活体红树植物幼苗的根部并向其地上组织传输的路径, 及其在内部组织中的最终分布情况。实验结果表明: (a) 经过 20d 的培养, 萘 (Na)、菲 (Phen)、An、Py 都从水培液迁移进入至 *Kc* 幼苗的根部, 并在蒸腾拉力的作用下传输至茎、叶等地上组织, 且四种 PAHs 迁移进入并在 *Kc* 幼苗内部传输的路径及其最终存在位置类似, 且四种 PAHs 在 *Kc* 幼苗内部组织中传输的速率顺序为 $Na > An$ 和 $Phen > Py$ 。四种 PAHs 胁迫对 *Kc* 幼苗根的生长都产生了抑制作用, 且其抑制作用大小的顺序为 $Na > An$ 和 $Phen > Py$, 其中 An 和 Phen 胁迫对 *Kc* 幼苗根生长的抑制作用差别不显著, 而 Na 对 *Kc* 幼苗根生长的抑制程度最为明显。(b) 经过 20d 的培养, 三种 PAHs (Na、Phen、Py) 都从水培液迁移进入至 *Ac* 幼苗的根部, 并在蒸腾拉力的作用下传输至茎部, 且三种 PAHs 迁移进入并在 *Ac* 幼苗内部传输的路径及其最终存在位置类似, 且三种 PAHs 在 *Ac* 幼苗内部组织中传输的速率顺序为 $Na > Phen > Py$ 。三种 PAHs 胁迫对 *Ac* 幼苗根的生长都产生了抑制作用, 且其抑制作用大小的顺序是 $Na > Phen > Py$, 其中 Na 对 *Ac* 幼苗根生长的抑制程度最为明显。

本文首次结合了 SSF 分析法、FM 和 TPLCSM 技术研究了典型 PAHs 在红树植物中的环境行为，为 PAHs 在红树林生态系统中的相关研究提供了新的手段、方法和研究思路。通过研究不同种类红树植物的叶、茎、根对典型 PAHs 的吸收、转运、降解等过程，初步揭示了红树植物对典型 PAHs 的植物修复、污染控制的过程、机制等。也为进一步研究 PAHs 污染胁迫下不同种类红树植物生长情况、生理生态及生化效应等奠定了研究方法基础。

关键词：原位研究；多环芳烃；红树植物；固体表面荧光分析法；荧光显微技术；双光子激光共焦扫描显微技术；环境行为

Abstract

Mangroves are the intertidal plant formations growing along the coastlines of tropical and subtropical regions. Due to an increase in population, and expansion of industrial agricultural areas and port, a large number of pollutants are discharging directly or indirectly into offshore marine areas. Human activities have seriously led to the damage of many mangroves. The unique features of mangrove ecosystems such as high primary productivity, abundant detritus, rich organic carbon and anoxic conditions make themselves a reservoir of polycyclic aromatic hydrocarbons (PAHs) from anthropogenic inputs. However, the published research on PAHs in the mangrove wetland has mainly focused on the analysis of concentrations of individual PAH and total PAHs compounds in the sediment; the determination of the distribution and proportion of PAHs in different tissues of many mangrove species; the evaluation of the possible sources of PAHs in the wetland; and the microcosm studies concerning the removal of PAHs from surface- or bottom-contaminated sediments. As a result, research on the direct uptake of PAHs by mangrove plants as the core of the wetland is rarely involved, nor are the related mechanisms being explained.

In this study, a systematic method was established for *in situ* study on the environmental behaviors of typical PAHs in the mangrove wetland utilizing the solid surface fluorimetry (SSF) and fluorescence microscopy (FM) as well as the combination of two photon laser confocal scanning microscopy (TPLCSM). And the environmental behaviors of typical PAHs in mangrove plants were studied *in situ* with the established methods. Main results were summarized as followings:

(1) A SSF approach combining with a laboratory-made solid substrate holder and a fluorescence spectrophotometer was established for direct determination of anthracene (An) adsorbed on the leaves of four mangrove species (*Avicennia marina* Am, *Bruguiera gymnorrhiza* Bg, *Kandelia candel* Kc, *Rhizophora stylosa* Rs). The experimental results were as follows: (a) With the same exposure time (90min) of An,

the leaves of the four mangrove species showed different capacities for adsorbing An, and within a certain range, the relative SSF intensities of the An adsorbed on the upper sides of leaves of the four mangrove species increased linearly with increments in the exposure quantity of An. The recoveries for the four species were between 70.2% and 110.8%, which met the requirement of practical applications. (b) In the same exposure concentration of An, the relative SSF intensities of leaves of the four mangrove species increased first and then decreased as the time progressed. And the maximum relative SSF intensities of the four species were in the order $Am > Kc > Rs > Bg$. (c) In the same experimental conditions, the relative SSF intensities of the adsorbed An on the upper side of the same mangrove leaves were much stronger than those adsorbed on the lower side, which has many stomata. Therefore, in addition to the uptake of PAH via waxy cuticle, the stomatal pathway was also important for the PAH entering into the mangrove leaves. Further studies showed that the relative SSF intensities in different regions of the same leaf differed from each other, and it provided us with very important information that the adsorbed An on the mangrove leaf surface might not “equally” penetrate the epidermal wax layer into the internal leaf tissues, but might have “priority” locations. However, the reasons were not clear.

(2) Based on the established SSF method, a synchronous solid surface fluorimetry (S-SSF) method combining with an optical fiber and a fluorescence spectrophotometer was established firstly for direct determination of the An and Pyrene (Py) which were adsorbed on the leaves of two mangrove species in single-component or in mixtures. The results indicated that the quantity of An and Py adsorbed on the mangrove leaves in single-component or in mixtures linearly correlated with their S-SSF intensities. And the recoveries of this S-SSF method for the leaves of two mangrove species were between 82.9% and 116.5%, which met the requirement of practical applications; Secondly, the distribution of An adsorbed on the living *Kc* leaves were studied *in situ* utilizing the established S-SSF approach, and

the GC-MS was also used to verify the accuracy of the S-SSF method. The experimental results demonstrated that in a certain period of exposure time of An, the trends of the quantity of adsorbed An on the upper side of *Kc* leaves determined by the two methods were similar. Thus, the S-SSF could be used as a novel *in situ* method for further studies. Finally, the photolysis of An and Py on the leaves of two mangrove species in single-component or in mixtures were directly studied by the S-SSF, and the results were as follows: (a) The processes of photolysis of An and Py in single-component adsorbed on the leaves of two mangrove species followed first-order reaction kinetics. And in the same experimental conditions, the higher the quantity of adsorbed PAH on the mangrove leaves, the slower the reaction rate of the photolysis of the PAH, moreover, the reaction rate of the adsorbed Py on the leaves of the same mangrove species was much slower than that of the same amount of adsorbed An. (b) The processes of photolysis of An and Py in mixtures adsorbed on mangrove leaves also followed first-order reaction kinetics. And to the same amount of PAH in mixtures, the reaction rate of PAH adsorbed on *Kc* was slower than that adsorbed on *Ac*. The experimental results also revealed that compared with the An and Py in single-component mode adsorbed on the same kind of mangrove leaves, the reaction rate of An in mixtures slowed down, while the reaction rate of Py in mixtures speeded up. (c) The processes of photolysis of An and Py in single-component or in mixtures, and in water followed first-order reaction kinetics. And to the same amount of PAH in single-component mode, the reaction rate of PAH in water was faster than that adsorbed on mangrove leaves. Compared with An and Py in single-component mode in water, the reaction rate of An in mixtures slowed down, while the reaction rate of Py in mixtures speeded up.

(3) Under the artificial simulating conditions, the An selected as the model PAH was applied as a homogeneous layer to the upper side of the living *Kc* leaves. During the exposure time of 96 hours, the SSF was firstly utilized for direct determination of the adsorbed An on the *Kc* leaves, then the FM was used to visualize how An was

taken up into *Kc* leaves and where it was located in the inner leaf tissues. The results demonstrated that the overall amount of An residing on the *Kc* leaf surface decreased gradually as time progressed. And after a certain period of exposure time, equilibration of the An might be achieved between the leaf surface and the atmosphere. And as the time passed, the An existing on the upper side of *Kc* leaves was observed moving from the upper epicuticular wax into the first and second hypodermis and upper palisade tissue, and the An existing on the lower side of *Kc* leaves moving from the stomata into the lower hypodermis and the lower palisade tissue, then ultimately into the spongy mesophyll. The results also showed that after exposed for 24 hours, there were several light blue dots in the upper and lower palisade tissues and spongy mesophyll, which might be the autofluorescence of An metabolites. Further studies also revealed that the residual quantity of An at the center was much less than that remaining at the edges of the same leaf surface after exposure of 24 hours, and this suggested that as time passed, the quantity of An retaining at the center of the leaf surface decreased more quickly, which might provide important new information about how An enters into *Kc* leaves.

(4) Under the artificial simulating conditions, the uptake, transportation and distribution of PAHs were *in situ* visualized in mangrove plants using TPLCSM, some results were as follows: (a) After 20 days of culture in contaminated solutions, the Naphthalene (Na), An, Phenanthrene (Phen) and Py had been observed entering into the roots of *Kc* seedlings and being transmitted to the stem and leaf due to transpiration. The pathways of the four PAHs from the culture solutions into the plants were similar, and the results also indicated that the transmission rates of the four PAHs in *Kc* seedlings were in the order $Na > An$ and $Phen > Py$. The growth of the root of *Kc* seedlings was inhibited by the PAHs and the inhibitional effect was in the order $Na > An$ and $Phen > Py$. In addition, the inhibition of An and Phen to the root growth was not significant difference, and the inhibition of Na was the most obvious. (b) After 20 days of culture in contaminated solutions, the three PAHs (Na, Phen, Py)

had been observed entering into the roots of *Aegiceras corniculata* (*Ac*) seedlings and being transmitted to the stem due to transpiration. The pathways of the three PAHs from the culture solutions into the plants were similar, and the results also indicated that the transmission rates of the three PAHs in *Ac* seedlings were in the order Na>Phen>Py. The growth of the root of *Ac* seedlings were inhibited by the PAHs and the inhibitional effect was in the order Na>Phen>Py, and the inhibition of Na was the most obvious.

In this study, the SSF, FM and TPLCSM were firstly combined for *in situ* study on the environmental behaviors of typical PAHs in the mangrove plants. It provided novel means, methods, and research idea for the related studies of PAHs in mangrove ecosystems. Through studying the processes of uptake, transportation and degradation of typical PAHs in different kinds of mangrove leaves, stem and roots, we revealed the processes and mechanisms on phytoremediation for typical PAHs and pollution control of mangrove plants. Moreover, it layed a methodological foundation for further study of the growth, ecophysiology and biochemical effects of different kinds of mangrove plants under the pollution stress of PAHs.

Key words: *in situ* study; polycyclic aromatic hydrocarbons; mangrove plant; solid surface fluorimetry; fluorescence microscopy; two photon laser confocal scanning microscopy; environmental behavior

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