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硕 士 学 位 论 文

原位定量和可视化研究红树植物根表面典型多环芳烃的截留行为

In Situ Visual and Quantitative Studies on Retention of Typical PAHs on Mangrove Root Surface

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缩略词表

缩略词	英文	中文
A. corniculatum	*Aegiceras corniculatum,*	桐花树
A．marina	*Avicennia marina*	白骨壤
Ant	Anthracene	蒽
B. gymnorhiza	*Bruguiera gymnorhiza*	木榄
B[a]P	Benzo[a]pyrene	苯并[a]芘
CNMs	Carbon nano-materials	碳质纳米材料
CNTs	Carbon nanotubes	碳纳米管
Fla	Fluoranthene	荧蒽
FLIM	Fluorescence lifetime imaging microscopy	荧光寿命成像显微
Flu	Fluorene	芴
FM	Fluorescence microscope	荧光显微镜
FOF	Fiber optic fluorimetry	光纤荧光法
GC	Gas chromatography	气相色谱
GO	Graphene oxide	氧化石墨烯
HPLC	High performance liquid chromatography	高效液相色谱
IFM	Inverted fluorescence microscope	倒置荧光显微镜
K. obovata	*Kandelia obovata*	秋茄
K_{ow}	Octanol-water partition coefficient	辛醇水分配系数
LC	Liquid chromatography	液相色谱
LCSM	Laser confocal scanning microscopy	激光共聚焦扫描荧光显微技术
LIF	Laser induced fluorimetry	激光诱导荧光法
LITRF	Laser induced nano-second time-resolved fluorescence technique	激光诱导纳秒时间分辨荧光光谱技术
MFSA	Microscopic fluorescence spectrometric analysis	显微荧光光谱分析
MWCNTs	Multi-walled carbon nanotubes	多壁碳纳米管
Nap	Naphthalene	萘
PAHs	Polycyclic aromatic hydrocarbons	多环芳烃
PCBs	Polychlorinated biphenyls	多氯联苯
Phe	Phenanthrene	菲

POPs	Persistent organic pollutants	持久性有机污染物
Pyr	Pyrene	芘
R. stylosa	*Rhizophors stylosa*	红海榄
S/N	Signal-to-noise	信噪比
SEM-EDX	Scanning electron microscopy with energy dispersive X-ray	能量色散 X 射线扫描电子显微镜
SFS	Synchronous fluorescence spectroscopy	同步荧光光谱技术
SPLCSM	Single- photon laser confocal scanning microscopy	单光子激光共聚焦扫描荧光显微技术
SSF	Solid surface fluorimetry	固体表面荧光法
TPLCSM	Two-photon laser confocal scanning fluorescence microscopy	双光子激光共聚焦荧光显微技术

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中文摘要

植物通过根部吸收土壤/沉积物中的多环芳烃（PAHs），是 PAHs 地球化学循环的重要组成部分，也是影响 PAHs 环境归趋的重要因素。红树植物（Mangrove）是热带、亚热带河口优势物种，其在维护海岸-河口的生态平衡及其海域的有机污染物净化有着极其重要的作用，其拥有特殊的气生根和支柱根结构，使得根部吸收 PAHs 不可忽视。目前，科研工作者围绕植物根部吸收 PAHs 过程、机制已开展了大量工作，然而随着显微技术发展，研究揭示 PAHs 的环境行为较宏观表面连续萃取方法有所不同，需从微尺度加以研究。

本文在综述红树林沉积物中 PAHs 污染及植被生态系统中 PAHs 原位方法研究的基础上，以 PAHs 为持久性有机污染物（POPs）的代表，利用实验室自行搭建具备原位微区定量和可视化 PAHs 环境行为潜力的显微荧光光谱分析（MFSA）技术，建立了红树植物典型 PAHs 原位微区定量和可视化方法，并在此基础上探讨了苯并[a]芘（B[a]P）、芘（Pyr）和蒽（Ant）在秋茄（*K. obovata*）侧根和主根表面的截留行为；其后，进一步探讨了新型碳质纳米材料（CNMs）氧化石墨烯（GO）对代表性红树植物 *K. obovata* 侧根和主根吸收过程中 B[a]P、Pyr 和 Ant 截留行为的影响。与此同时，在 GO 与典型 PAH 共存时，初步探讨了盐度对 *K. obovata* 根表面 B[a]P、Pyr 和 Ant 截留行为的影响。这从微区尺度为探讨植物根部吸收 PAHs 行为及研究提供了原位定量、可视化方法。

（1）利用典型 PAH Ant 作为模式化合物，以新鲜的红树植物 *K. obavata* 和白骨壤（*A. marina*）的根为基质，利用 MFSA 系统建立了直接吸附于 *K. obavata* 和 *A. marina* 根表面 Ant 的微区测定和可视化新方法。实验结果表明，吸附于 2 种红树根表面微区中 Ant 的量与其表面微区相对荧光强度之间具有较好的线性关系。所建方法线性范围分别为 5.3-63.2 和 10.5-52.6 pg/μm^2，检出限分别为 1.1 和 5.5 pg/μm^2，相对标准偏差均小于 12.5%（n=9），加标回收率分别为 98.1-117.0% 和 81.2-110.9%。揭示搭建的 MFSA 系统具备原位获取植物根表面微区 Ant 荧光光谱定量信息和荧光图像信息的能力。

（2）基于上述研究工作，在实验室模拟生态条件下，利用 MFSA 系统建立了红树植物 *K. obovata* 侧根和主根表面微区中 B[a]P、Pyr 和 Ant 原位测定和可视

化分布方法，并利用所建方法探讨了 *K. obovata* 侧根和主根表面 B[a]P、Pyr 和 Ant 截留行为及其主要影响因素。实验结果表明：（a）*K. obovata* 侧根和主根表面微区中 B[a]P、Pyr 和 Ant 的截留量与微区相对荧光强度之间均具有较好的线性关系，且所建方法的检测限分别为 44.2，59.7 和 36.3 ng/g（侧根），主根分别为 42.8，62.4 和 39.1 ng/g；（b）*K. obovata* 侧根和主根表面的截留的 B[a]P、Pyr 和 Ant 均呈非均匀分布，且侧根与主根截留量差异显著（侧根>主根，p <0.05），揭示这与 *K. obovata* 的被动和主动吸收 PAH 及根表面的极性指数相关。

（3）在模拟生态条件下，初步探讨了典型 CNMs GO 对 *K. obovata* 根表面微区中 B[a]P、Pyr 和 Ant 截留行为的影响；其后，进一步探讨盐度对 GO 与 PAH 共存时，*K. obovata* 侧根和主根表面微区中 B[a]P、Pyr 和 Ant 截留行为的影响。结果揭示，GO 提高 B[a]P、Pyr 和 Ant 在 *K. obovata* 侧根和主根表面微区中截留量，均呈现 Ant < Pyr < B[a]P；GO 也可提高 B[a]P、Pyr 和 Ant 在根中的输入水平，揭示这与 PAHs-GO 相互作用和 GO-PAHs 共同暴露改变根表面膜渗透性有关。盐度升高 *K. obovata* 侧根和主根表面微区中 B[a]P、Pyr 和 Ant 截留量减少，其有利于 PAHs 进入根的内部。

关键词：原位；MFSA；红树；根表面微区；PAHs；GO；截留

Abstract

There are over 90% of PAHs occuring in the surface of sediment/soil in the environment. The uptake of PAHs by plant roots is an important part of the geochemical cycle of organic contaminants and also is an important factor affecting the migration and fate of PAHs in the environment. Mangrove is the dominant species of plants that were located in tropical and subtropical estuary, and plays an extremely important role to cleanup organic pollutants and maintain balance of inshore coast ecosystem. Owing to aerial roots and prop root of mangrove plant interspersed in the sediments/soil, the uptake of PAHs by roots can not be neglected. Numerous researchers have focused on the uptake of PAHs by plant root and its mechanisms. However, with the development of fluorescence microscopic techniques, it has been observed that the environmental behavior (including distribution and retention) of PAHs at macro-scale differs from that obtaind by sequential extraction techniques at micro-scale, and thus the uptake of PAHs by plant root needs to be further conducted at micro-scale.

Based on the reviews on contamination of PAHs in mangrove sediments and *in situ* analytical methods of investigating PAHs in plant ecosystem, the MFSA, which has the potential to *in situ* determine and visualize the PAHs on the surface micro-zone, was used to establish a method for *in situ* quantification and visualization of the B[a]P, Pyr and Ant on the surface micro-zone of mangrove root. Then, retention of the B[a]P, Pyr and Ant on the *K. obovata* lateral root and taproot surface micro-zone were investigated *in situ* using the established method. Also, retention of the B[a]P, Pyr and Ant on the micro-zone of mangrove *K. obovata* lateral root and taproot surface were also studied in the presence of GO, know as a typical two-dimension CNMs. Last, the implications of salinity on retention of the B[a]P, Pyr and Ant on surface micro-zone of mangrove lateral root and taproot were investigated *in situ* in the present of GO. There is a novel *in situ* quantitative and visualization method provided for investigating the uptake of PAHs by plant root surface at micro scale.

(1) The model PAH Ant and two kinds of fresh mangrove roots (*K. obovata* and *A. marina*) were selected to establish a novel method for *in situ* quantifying and visualizing PAHs adsorbed on root surface micro-zone using MFSA system. The

results showed that there were good linear relationships between the amounts of Ant adsorbed on the surface micro-zone of the roots and their relative fluorescence intensities. The dynamic linear ranges of the established method were 5.3-63.2 and 10.5-52.6 pg/μm^2, with the detection limits of 1.1 and 5.5 pg/μm^2. The relative standard deviations of the established method were less than 12.5% (n=9), and the recoveries were 98.1-117.0% and 81.2-110.9%. Above results revealed that the MFSA system (set in the lab) has the ability to obtain quantitative information and fluorescence images of the PAH on surface micro-zone.

(2) Based on above work, the MFSA system was used to establish the new method that *in situ* determine and visualize the B[a]P, Pyr and Ant on surface micro-zone of *K. obovata* lateral root and taproot in the simulated ecological conditions. Then, retention of individual B[a]P, Pyr and Ant on the surface micro-zone of *K. obovata* lateral root and taproot were investigated *in situ*. The results indicated that: (a) there existed good linear relationships between the amounts of B[a]P, Pyr and Ant on the surface micro-zone of the *K. obovata* lateral root and taproot and their relative fluorescence intensities. The detection limits of the proposed method were 44.2 ng/g for B[a]P, 59.7 ng/g for Pyr, and 36.3 ng/g for Ant (lateral roots), and also the taproot roots were 42.8 ng/g for B[a]P, 62.4 ng/g for Pyr and 39.1 ng/g for Ant; (b) Retention of B[a]P, Pyr and Ant on the surface micro-zone of the *K. obovata* lateral root and taproot had significant difference ($p < 0.05$); there were also uneven distribution of B[a]P, Pyr and Ant both for the *K. obovata* lateral root and taproot, which were related to the uptake pattern of the the B[a]P, Pyr and Ant (passive or active uptake) and the surface polarity of the *K. obovata* root surface.

(3) In the simulated ecological condition, the retention of the B[a]P, Pyr and Ant on the surface micro-zone of *K. obovata* lateral root and taproot in the present of GO were investigated *in situ* by the proposed method. The results showed that the GO improved the retained amounts of the B[a]P, Pyr and Ant on surface micro-zone of the *K. obovata* lateral root and taproot with the order of Ant < Pyr < B[a]P. Also, it has been observed that GO improved the amounts of B[a]P, Pyr and Ant in the *K. obovata* root tissue, which were related to the interaction of the individual B[a]P, Pyr and Ant with GO and also the effects of the co-exposure of GO and the PAH on the permeability of the root surface of *K. obovata* root. The salinity (represented by KCl) decreased the amounts of retention of the B[a]P, Pyr and Ant on surface micro-zone of *K. obovata* lateral root and taproot by improving the access of the individual B[a]P,

Pyr and Ant into root tissue, being related to the active uptake of the B[a]P, Pyr and Ant by *K. obovata* root.

Key words: *In situ*; MFSA; mangrove; root surface micro-zone; PAHs; GO; retention