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硕 士 学 位 论 文

烷基菲毒性效应及腐植酸对其生物有效性影响初探

Study on toxic effect of alkyl phenanthrenes and the influence of humic acid on the bioavailability of them

刘 洋 之

指导教师姓名：张 勇 教 授
专 业 名 称 ：环 境 科 学
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摘 要

烷基取代多环芳烃（Alkyl Polycyclic Aromatic Hydrocarbons, Alkyl PAHs）作为环境中 PAHs 的重要组成部分，因其毒性效应强、种类繁多，且与母环 PAHs（Parent Polycyclic Aromatic Hydrocarbons, Parent PAHs）致毒机理差异显著已逐步成为研究热点。然而，多数毒理学研究忽略了实际环境因素对烷基取代 PAHs 生物有效性的影响。本研究首先从形态学指标（心脏拉长、心率、孵化率和孵化时间）、生化指标（SOD、CAT 活力及 MDA 水平）两个层面探讨了母环 PAHs 与烷基取代 PAHs 毒性效应差异。其次，为深入了解环境因素对烷基取代 PAHs 环境行为的影响，用激光诱导纳秒时间分辨荧光（Laser-Induced Nanosecond Time Resolved Fluorescence, LITRF）系统结合荧光猝灭法研究腐植酸（Humic Acid, HA）与烷基取代 PAHs 及母环 PAHs 相互作用，并以探讨了 HA 对烷基取代 PAHs 生物有效性的影响。具体研究结果如下：

（1）形态学指标表明菲（Phenanthrene, Phe）、3-甲基菲（3-methyl Phenanthrene, 3-MP）和 9-乙基菲（9-ethyl Phenanthrene, 9-EP）对海洋青鳉鱼（*Oryzias melastigma*）胚胎均具有一定的致畸效应并不同程度影响胚胎的死亡率和孵化率等，3-MP、9-EP 在发育过程中可显著影响胚胎心率及心脏发育水平。相较于 3-MP、9-EP，Phe 对静脉窦-动脉球（Sinus Venosus-Bulbus arteriosus, SV-BA）的拉长效应较低，表明烷基取代 PAHs 可显著影响海洋青鳉鱼胚胎心脏功能。SOD 和 CAT 两种酶对三种 PAHs 暴露均呈现先诱导后抑制趋势。实验结果表明经 5 天暴露后，各 PAHs 处理组 MDA 水平显著高于对照组。当 3-MP 和 9-EP 暴露浓度为 3.0×10^{-6} mol L^{-1} 时，MDA 水平达到最高值，分别为对应浓度 Phe 处理组 129.8%、144.4%，11 天时，3-MP 和 9-EP 各浓度处理组 MDA 水平均显著高于对应 Phe 处理组，3-MP 暴露浓度为 3.0×10^{-6} mol L^{-1} 时，MDA 水平达到最高值，为对应 Phe 处理组 115.5%。

（2）以 LITRF 系统结合荧光猝灭法原位研究 HA 分别与 Phe、9-EP 和惹稀（Retene, Ret）相互作用。利用 Freundlich 非线性等温吸附模型描述 Phe、9-EP 和 Ret 与 HA 结合特性，LITRF 猝灭法与传统荧光法获得的模型拟合参数及单点结合系数一致。其中，参数 n 小于 1，表明 Phe、9-EP 及 Ret 与 HA 均以非线性

形式结合，且相同给定平衡浓度下，HA 与 9-EP 和 Ret 单点结合系数 K_{OC} 大于 Phe，而 9-EP 和 Ret 结合能力相近，且均随给定浓度增加而降低。疏水性、取代基及与 HA 疏水空腔适应能力决定特定 PAHs 与 HA 结合特性。荧光寿命分析结果表明，HA 存在下 Phe、9-EP 和 Ret 寿命分别为 36.90、35.34 和 35.13 ns，与未加入 HA 时的 36.36、35.34 和 35.84 ns 无明显差异，表明 Phe、9-EP 和 Ret 与 HA 间的荧光猝灭以静态过程为主。

（3）以海洋青鳉鱼胚胎内 PAHs 富集量及 MDA 水平为效应指标，研究了 HA 对母环 PAHs 及烷基取代 PAHs 生物有效性的影响。研究表明，HA 可显著降低游离态 PAHs 浓度进而降低其生物富集和毒性效应。通过生物有效性模型拟合可知结合态 Phe、3-MP 及 9-EP 对生物富集的贡献率 α 分别为 15.00±1.69%~14.04±1.27%、17.17±2.36%~16.34±2.22%、24.32±2.71%~19.20±1.65%，其对毒性效应的贡献率 β 分别为 13.41±1.28%~11.94±2.16%、12.33±2.74%~10.61±3.20%、10.36±2.23%~8.93±1.73%。对于添加了 HA 的 PAHs 处理组而言，其 β/α 均小于 1，表明结合态 PAHs 对于生物富集效应贡献率更高。此外，β/α 与 logK_{OW} 间相关性分析结果表明 HA-PAHs 复合物的生物有效性与 PAHs 理化属性显著相关。

（4）采用同步荧光光谱法结合人工神经网络（Artificial Neural Network, ANN）和支持向量回归（Support Vector Regression, SVR）模型对荧光光谱重叠的 Phe 和 2-乙基菲（2-Ethyl Phenanthrene, 2-EP）进行同时测定。通过三维同步荧光法结合平行因子（Parallel Factor, PARAFAC）分析寻得 Phe 和 2-EP 特征波长差为 118 nm，在 220-280 nm 范围内，以 31 个波长处荧光强度值作为模型的输入特征变量，建立 ANN 和 SVR 模型，采用 17 个训练样本来预测 3 个待测人工合成样本中 Phe 和 2-EP 浓度。实验结果显示，ANN 模型分析 Phe 和 2-EP 预测样本回收率分别为 92.47%~104.90%和 96.14%~104.29%；SVR 模型分析预测样本回收率分别为 98.19%~101.27%，94.87%~104.18%。对 ANN 和 SVR 模型性能进行比较，表明 SVR 模型较 ANN 模型预测结果更好，SVR 模型用于实际水体样的测定，亦取得较为满意结果。

关键词：多环芳烃；烷基取代多环芳烃；氧化胁迫；生物有效性；海洋青鳉鱼；腐植酸

Abstract

Alkyl polycyclic Aromatic Hydrocarbons (Alkyl PAHs), an important component of PAHs in the environment, has gradually became the research focus for its variety, high toxicity and unique toxic mechanism. However, most toxicology studies ignored the effects of realistic environmental factors on the bioavailability of Alkyl PAHs. The study first discussed the toxicity differences of parent PAHs and alkyl PAHs in marine medaka (*Oryzias melastigma*) at two different levels: morphology level (heat stretching/rate, hatching rate/time) and biochemical level (vitality of SOD, CAT and level of MDA). Then, for further studying the effect of environmental factors on the environmental behavior of alkyl PAHs, the study used Laser induced nanoseconds time-resolved fluorescence (LITRF) to detect the interactions of phenanthrene (Phe), alkyl phenanthrenes (Alkyl Phes) and humic acid (HA). Lastly, the study reviewed the influence of HA on the bioaccumulation of the three PAHs and the levels of methane dicarboxylic aldehyde (MDA) with the presence of HA, in hope of laying theoretical foundations for research on toxicity and bioavailability to PAHs. The results are as followed:

(1) The morphology data of marine medaka showed that Phe, 3-methyl phenanthrene (3-MP) and 9-ethyl phenanthrene (9-EP) all had teratogenicity on the embryo and could influece the mortality rate and hatching rate of embryo to different extends. Compared with 3-MP and 9-EP, Phe had a low elongating effect on sinus venosus to bulbus arteriosus (SV-BA), which indicated that alkyl PAHs could significantly influence the cardiac development of medaka embryos. The activity of SOD and CAT showed the tendency of first inducing and then inhibiting when exposed to PAHs. Furthermore, for 5 d exposure, the level of MDA in every PAHs treatment group was significantly higher than that of control group. The concentration of MDA reached its highest level at 3.0×10^{-6} mol L^{-1} PAHs exposure groups, and MDA levels of 3-MP and 9-EP treatment groups were equal to 129.8% and 144.4% of that of Phe. After 11 d exposuring, the MDA level of all alkyl PAHs treatment groups,

excepting the highest exposure group of 9-EP in which all embryos were died, was significantly higher than that of Phe. And the MDA level reached the highest level at 3.0×10^{-6} mol L^{-1} 3-MP exposure groups, which was correspond to 115.5% of that of Phe.

(2) Investigation of the interactions between parent PAHs and alkyl PAHs, exemplified by Phe, 9-EP, retene (Ret), and HA was applied using LITRF. The binding characteristics of the dissolved HA and Phe, 9-EP and Ret were described by Freundlich nonlinear isothermal model. The results of the model parameters and the single point binding coefficients K_{OC} of Phe, 9-EP and Ret with HA via LITRF quenching method were consistent with those of the conventional fluorescence quenching method. The value of parameter n was less than 1, which showed the nonlinear bindings of Phe, 9-EP and Ret to HA, and the degree of nonlinearity of Phe was lower than 9-EP and Ret. At the same given equilibrium concentration, K_{OC}-the single point binding parameter of HA with 9-EP and Ret each, outnumbered Phe, while the binding ability of 9-EP and Ret were similar, and the binding affinity of the three PAHs with HA all decreased with the increase of equilibrium concentration.The binding characteristics of PAHs with HA largely depended on the their hydrophobicity, substituent groups and their ability to fit into hydrophobic cavities in HA. Fluorescence lifetime analysis showed the lifetimes of Phe, 9-EP and Ret in the presence of HA were 36.90, 35.34 and 35.13 ns. There was no big difference with the value of 36.36, 35.34 and 35.84 ns without HA, which indicated the quenching mechanism between HA with Phe, 9-EP and Ret each was primarily static quenching.

(3) This paper taken marine medaka embryos as the model, and the bioaccumulation of PAHs along with MDA levels as the effect target and discusses the influences of HA onto the bioavailability of parent PAHs and alkyl PAHs. Study showed HA could largely decrease free PAHs concentration and further reduce the bioavailability and toxic effect of PAHs. It could be concluded from fitting of bioavailability model that the contribution rates of HA-bound Phe, 3-MP and 9-EP to bioaccumulation (α) were 15.00±1.69%~14.04± 1.27%, 17.17±2.36%~16.34±2.22%, 24.32±2.71%~19.20±1.65%, and their contribution rates to toxicity (β) were

13.41±1.28%~11.94±2.16%, 12.33±2.74~ 10.61±3.20%, 10.36±2.23%~8.93±1.73%. To all PAHs treatment groups with HA, the β/α value were less than 1, indicating that HA-bound PAHs contributed more to bioaccumulation. In addition, the correlation analysis between β/α and log*K*ow showed that the bioaccumulation of HA-bound PAHs was hugely correlated with physical and chemical characters of PAHs.

(4) This study combined synchronous fluorescence spectroscopy with artificial neural network (ANN) and support vector regression (SVR) to determine the contents of Phe and 2-Ethyl phenanthrene (2-EP) which fluorescence spectra were overlapped in a binding system at the same time. It could be found that the characteristic wavelength deviation was 118 nm through three dimensional synchronous fluorescence spectroscopy combined with parallel factors analysis. Within the limits between 220~280 nm, experiment taked the fluorescence intensity of 31 different wavelength point as the input characteristic vector of the model and built ANN and SVR model to pre-evaluate the concentration of Phe and 2-EP in 3 synthetic samples to be tested using 17 training samples.Result showed that the recovery rates of these samples were 92.47%~104.90% and 96.14%~104.29% respectively based on the analysis of Phe and 2-EP with ANN model, while the recovery rates were 98.19%~101.27% and 94.87%~104.18% with SVR model analysis. Contrast between ANN and SVR model performance showed that SVR model's performance of predicting excels. Also the application of SVR model into real water gained a satifactory result.

Keywords: Polycyclic Aromatic Hydrocarbons; Alkyl Polycyclic Aromatic Hydrocarbons; Oxidative Stress; Bioavailability; Marine medaka; Humic Acid

缩略词表

缩略词	英文	中文
Alkyl PAHs	Alkyl Polycyclic Aromatic Hydrocarbons	烷基取代多环芳烃
Parent PAHs	Parent polycyclic Aromatic Hydrocarbons	母环多环芳烃
LITRF	Laser-induced Nanosecond Time Resolved Fluorescence	激光诱导纳秒时间分辨荧光
HA	Humic Acid	腐植酸
Phe	Phenanthrene	菲
3-MP	3-Methyl Phenanthrene	3-甲基菲
9-EP	9-Ethyl Phenanthrene	9-乙基菲
PARAFAC	Parallel Factor	平行因子
DLCs	Dioxin-like compounds	类二恶英化合物
TCDD	Tetrachlorodibenzo-p-dioxin	2,3,7,8-四氯二苯并二恶英
DOM	Disolved Organic Mater	溶解性有机质
EC_{50}	Semi Effect Concentration	半效应浓度
LC_{50}	Semi Lethal Concentration	半致死浓度
QSAR	Quantitative Structure Activity Relationship	定量构效关系模型
QSPR	Quantitative Structure Property Relationship	结构-性质相关模型
BLM	Biotic Ligand Model	生物配体模型
FIAM	Free Ion Activity Model	自由离子活度模型
SV-BA	Sinus Venosus-Bulbus Arteriosus	静脉窦-动脉球
LITRF	Laser Induced Nanosecond Time-Resolved Fluorescence	激光诱导纳秒时间分辨荧光
SOD	Superoxide Dismutase	超氧化物歧化酶
CAT	Catalase	过氧化氢酶
MDA	Methane Dicarboxylic Aldehyde	丙二醛
ROS	Reactive Oxide Species	活性氧自由基
ANN	Artificial Neural Network	人工神经网络
SVR	Support Vector Regression	支持向量回归