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# 砷-菲对蜈蚣草根部分不同碳基团的影响

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**摘要:** 采用离体鲜根实验和固态<sup>13</sup>C核磁共振技术(<sup>13</sup>C-NMR)研究蜈蚣草根部分对砷和菲的吸收特征及根部碳基团的影响作用。结果表明, 蜈蚣草根部分在无蒸腾作用的条件下能够有效地吸收砷和菲, 添加菲能够促进离体鲜根对砷的吸收, 添加砷也可提高菲的吸收, 与对照相比, 菲提高了 15%~53%。蜈蚣草离体鲜根中以烷氧基 C 为主, 羧基 C 比例最低, 且主要为羧酸、酯和酰胺类羧基 C, 添加砷和菲会显著增加蜈蚣草离体鲜根中羧基 C 的比例。蜈蚣草离体鲜根为应对砷和菲的胁迫形成了分子结构更为稳定和复杂的芳香类有机质, 从而提高离体鲜根的抗性和适应性。

**关键词:** 菲; 砷; 蜈蚣草; 固态<sup>13</sup>C核磁共振; 生物降解

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## Influence of Arsenate and Phenanthrene on Carbon-groups of *Pteris vittata* L. Roots

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**Abstract:** To ascertain absorption of arsenate and phenanthrene as well as their influence on carbon groups in excised roots of *Pteris vittata* L., the chemical structure of the carbon groups in excised roots was characterized by solid state <sup>13</sup>C-Nuclear Magnetic Resonance (<sup>13</sup>C-NMR). The results showed that the excised roots could effectively absorb As and PHE without transpiration, and PHE promoted As accumulation in the roots. Similarly, arsenate increased the adsorption of PHE by the excised roots, the concentration of PHE was increased by 15%-53% compared with CK. The carbon groups of the excised roots were dominated by O-alkyl C, the percentage of carboxyl C was the lowest, mainly composed of carboxylic acids, esters and amides. With the addition of As and PHE, the percentage of carboxyl C increased significantly. The more stable and complex aromatic organic matter was formed to improve the resistance and adaptability in excised roots of *Pteris vittata* L. under As and PHE stress.

**Key words:** phenanthrene; arsenic; *Pteris vittata* L.; solid-state <sup>13</sup>C-NMR; biodegradation

多环芳烃 (polycyclic aromatic hydrocarbons, PAHs) 和砷 (As) 是环境中普遍存在的有毒污染物, 具有高毒性、致癌、致畸作用, 并且两者共存会改变生物的基因表达并加大致癌风险<sup>[1,2]</sup>。PAHs 和 As 在人类工业活动过程中会释放到环境并在土壤中累积形成复合污染。Elgh-Dalgren 等<sup>[3]</sup>对木材加工厂调查发现, 土壤中 PAHs 和 As 含量分别高达 46 mg·kg<sup>-1</sup> 和 105 mg·kg<sup>-1</sup>。朱岗辉等<sup>[4]</sup>对我国南部典型工业场地调查发现, PAHs 与 As 复合污染较普遍, 其中, 采煤厂主要存在 As 和 PAHs 污染, 超标率分别为 87.5% 和 75.0%。Sun 等<sup>[5]</sup>研究证实, 蜈蚣草是治理 As 和 PAHs 复合污染的优选植物, 它不仅对 As 有较好的富集能力, 同时对 PAHs 具有较强的累积作用, 地上部累积可超过 1 000 μg·kg<sup>-1</sup>。

<sup>13</sup>C-核磁共振 (<sup>13</sup>C-NMR) 波谱能在不改变植物原样结构的基础上对其有机质的碳结构骨架进行定

性和定量分析。Ahmad 等<sup>[6]</sup>应用<sup>13</sup>C-NMR技术研究表明, 土壤有机物的碳基团能显著影响其对有机污染物的吸附作用, 芳香碳是影响土壤有机物对非离子型杀虫剂吸附作用的关键因素。<sup>13</sup>C-NMR具有可以直接测量碳骨架、化学位移范围宽、信息量大等优点, 近年来开始应用于有机质结构对污染物吸附影响的研究<sup>[7,8]</sup>。

离体鲜根吸收污染物实验可用于研究植物根系对污染物的吸收特征与机制。Cheng 等<sup>[9]</sup>利用两种红树属植物离体鲜根研究了根木质化/木栓化对红树林吸收和耐受铅 (Pb) 的影响, 结果表明根部木质化/木栓化可以有效限制植物根系对 Pb 的吸收和积

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累. Su 等<sup>[10]</sup>开展水稻离体鲜根对有机污染物的吸收机制研究发现,水稻根系对极性有机污染物的吸收主要通过共质体途径,对类似于 PHE 的非极性有机污染物则通过质外体途径吸收. 本研究采用蜈蚣草离体鲜根吸收 As 和 PAHs 实验,探索蜈蚣草根部分吸收两种污染物的特征规律,并借用固态<sup>13</sup>C-NMR 技术检测蜈蚣草根部分不同有机基团,探明不同碳基团与 As 和 PAHs 吸收的相互关系.

## 1 材料与方 法

### 1.1 实验设计

将采自湖南郴州市的蜈蚣草孢子均匀撒在播种盘基质中(花卉土:蛭石=1:1),洒水保持湿润,覆薄膜保湿,置于温室内培育,室内昼间温度 25℃,夜间温度 20℃,湿度 65%. 当孢子发育出数片幼叶时,分苗移栽到 1/5 强度的霍格兰营养液(pH=6.0)中进行培养. 5 个月后,选取长势优良且基本一致的蜈蚣草,在流动的自来水下将蜈蚣草根部分洗净,再用去离子水冲洗 3 遍. 随后将洗净的鲜根剪成 1~2 cm 段备用,放置于湿润的纱布间防止其在称量过程中失水.

本实验共设置 8 组处理:空白对照(CK)、单独加砷(As)、单独加菲(PHE)、砷加菲(As+PHE)、单独加菲降解菌(产碱杆菌 *Alcaligenes* sp., B)、菲降解菌加砷(As+B)、菲降解菌加菲(PHE+B)、菲降解菌加砷和菲(As+PHE+B). 实验中砷选取砷酸钠( $\text{Na}_2\text{HAsO}_4 \cdot 7\text{H}_2\text{O}$ ),五价砷的配置浓度为  $10 \text{ mg} \cdot \text{L}^{-1}$ ,菲的配置浓度为  $2 \text{ mg} \cdot \text{L}^{-1}$ . 称取 5 g 鲜根样品置于含有不同处理营养液的锥形瓶中,溶液体积为 30 mL,恒温振荡 24 h( $37^\circ\text{C}$ ,  $150 \text{ r} \cdot \text{min}^{-1}$ ),每组处理设置 3 个重复. 振荡结束后将鲜根收获,选取一部分鲜根  $105^\circ\text{C}$  杀青 30 min,  $65^\circ$  烘干 24 h,测定干重,用于总砷测定. 剩余部分鲜根冷冻干燥 24 h,冰浴磨碎于  $-80^\circ\text{C}$  冷藏,用于菲含量和碳基团等测定. 振荡前后培养液均留样保存,并测定其中的总 As 和 PHE 的浓度.

### 1.2 总砷含量的测定

将烘干的鲜根样品采用  $\text{HNO}_3\text{-HClO}_4$  (US EPA 3050)方法消煮,定容稀释后,用原子荧光光度计(AFS-9130)测定总 As 含量. 分析过程所用试剂均为优级纯(GR),并采用国家标准参比物质(GBW-07603)作为分析质量控制的标准<sup>[5]</sup>.

### 1.3 菲含量的测定

将溶液样品和冰浴磨碎后的鲜根样品,均用丙

酮和二氯甲烷混合溶液(体积比 1:1)超声萃取 3 次,上层萃取液旋转蒸发浓缩至约 0.5 mL,转移到硅胶柱中净化,并用正己烷和二氯甲烷混合液淋洗,承接的溶液旋转蒸发至约 1 mL,加入 7~8 mL 正己烷,继续旋转蒸发至约 0.5 mL,加内标后定容至 1 mL. 采用 GC-MS(Agilent 6890)测定 PHE 的含量( $n=3$ ,  $\text{RSD} < 10.58$ )<sup>[5]</sup>.

### 1.4 <sup>13</sup>C-NMR 测定

蜈蚣草根的<sup>13</sup>C-NMR 波谱用瑞士布鲁克 AVANCE III 400 MHz 型固体核磁共振仪检测. 采用交叉极化-边带全抑制魔角旋转技术,转子直径为 4 mm,光谱频率为 100.37 MHz、旋转频率为 5 000 Hz,接触时间为 3 ms、循环延迟时间为 1 s,采样次数为 6 000 次. 谱图的化学位移用外标甘氨酸的羰基碳(176.03 ppm)校正.

### 1.5 数据与图谱处理

数据采用 SPSS 21.0 (SPSS Inc., Chicago) 软件进行方差分析与显著性分析,均值差的显著水平为 0.05;并用 Origin 8.6 软件绘制图表;蜈蚣草鲜根样品的<sup>13</sup>C核磁共振波谱图采用 Peakfit 4.0 分峰软件进行分峰解叠.

## 2 结果与分析

### 2.1 蜈蚣草离体鲜根中砷的含量

在无蒸腾作用条件下,蜈蚣草离体鲜根对砷表现出较强的吸收和累积作用(见表 1). 在添加砷的处理条件下,添加的菲和菲降解菌促进蜈蚣草离体鲜根吸收砷,不同处理中砷含量由大到小顺序为:  $\text{As} + \text{PHE} + \text{B} > \text{As} + \text{PHE} > \text{As} + \text{B} > \text{As}$ ,其中,  $\text{As} + \text{PHE} + \text{B}$  处理中的砷含量比单独砷处理提高了 10%. 在不添加砷的处理组中蜈蚣草离体鲜根和培养液中均检出砷,这可能是由于前期育苗土壤和培养液药品中砷带入引起的.

### 2.2 蜈蚣草离体鲜根对菲的吸收

蜈蚣草离体鲜根对菲具有一定的耐受性,并且能够吸收和累积一定含量的菲(见表 2). 添加砷和菲降解菌,有助于促进蜈蚣草离体鲜根对菲的吸收和积累,各处理间蜈蚣草离体鲜根中菲含量为  $\text{As} + \text{PHE} + \text{B} > \text{As} + \text{PHE} > \text{PHE} + \text{B} > \text{PHE}$ ,但前 3 处理之间并无显著性差异,不过均显著高于单独菲处理. 与不添加砷相比,添加砷后蜈蚣草离体鲜根中的菲含量显著升高,菲含量是不添加砷的 1.15~1.53 倍. 在不含砷的条件下,添加菲降解菌能够显著促进蜈蚣草离体鲜根对菲的吸收;而当培养液中加砷

后,菲降解菌对蜈蚣草离体鲜根吸收菲的促进作用不显著.蜈蚣草根中累积菲的量非常低,为  $47.5 \times 10^{-3} \sim 78.7 \times 10^{-3} \mu\text{g}$ ,占菲总去除量的 0.1% 左右,

表明蜈蚣草吸收作用对于菲的去除贡献率很小,可能是挥发、光解及菲降解菌的降解作用对培养液中菲的去除起主要作用.

表 1 蜈蚣草离体鲜根对砷的富集情况<sup>1)</sup>

Table 1 Arsenate accumulation in the excised roots of *P. vitatta* L.

处理组	离体鲜根(DW) /mg·kg <sup>-1</sup>	处理前培养液 /mg·L <sup>-1</sup>	处理后培养液 /mg·L <sup>-1</sup>
CK	0.041 ± 0.02 d	0.031 ± 0.04	0.018 ± 0.02 c
B	0.070 ± 0.05 d	0.043 ± 0.01	0.023 ± 0.03 c
As	142.75 ± 2.98 c	9.40 ± 0.51	5.76 ± 0.67 a
PHE	0.027 ± 0.03 d	0.033 ± 0.01	0.047 ± 0.06 c
As + B	145.66 ± 2.24 c	8.77 ± 1.17	5.24 ± 0.31 b
PHE + B	0.053 ± 0.02 d	0.057 ± 0.04	0.063 ± 0.03 c
As + PHE	149.38 ± 9.30 b	8.56 ± 0.48	5.34 ± 0.31 b
As + PHE + B	155.80 ± 2.93 a	8.66 ± 0.63	5.12 ± 0.06 b

1) 不同小写字母表示各处理之间存在显著性差异 ( $P < 0.05$ )

表 2 蜈蚣草离体鲜根对菲的吸收和累积情况<sup>1)</sup>

Table 2 Phenanthrene uptake and accumulation in the excised roots of *P. vitatta* L.

处理组	离体鲜根(DW) /μg·kg <sup>-1</sup>	处理前培养液 /mg·L <sup>-1</sup>	处理后培养液 /mg·L <sup>-1</sup>	菲累积量 × 10 <sup>-3</sup> /μg
CK	—	—	—	—
B	—	—	—	—
As	—	—	—	—
PHE	214.50 ± 63.61 b	2.23 ± 0.49	1.47 ± 0.10 a	47.53 ± 13.4 b
As + B	—	—	—	—
PHE + B	310.53 ± 39.66 a	2.17 ± 0.31	0.095 ± 0.01 bc	69.27 ± 9.86 a
As + PHE	328.67 ± 17.39 a	2.12 ± 0.12	0.152 ± 0.13 b	73.92 ± 3.34 a
As + PHE + B	358.13 ± 66.03 a	1.97 ± 0.08	0.031 ± 0.01 c	78.75 ± 15.26 a

1) “—” 表示忽略不计或未检出,不同小写字母表示各处理之间存在显著性差异 ( $P < 0.05$ )

### 2.3 蜈蚣草鲜根的固态<sup>13</sup>C 核磁共振波谱分析

根据已有研究对<sup>13</sup>C-NMR波谱中有机物主要碳基团化学位移的分析<sup>[11-13]</sup>,有机物的碳化学位移主要分为 4 部分:烷基碳区(0 ~ 50 ppm)、

烷氧基碳区(50 ~ 110 ppm)、芳香碳区(110 ~ 160 ppm)和羰基碳区(160 ~ 220 ppm).<sup>13</sup>C-NMR波谱中具体功能区峰位碳基团的化学位移情况如表 3 所示<sup>[14-17]</sup>.

表 3 <sup>13</sup>C-NMR图谱中主要功能区峰位 C 基团归属

Table 3 Carbon groups distribution in solid-state <sup>13</sup>C-NMR spectra

化学位移范围/ppm	碳基团归属
0 ~ 50	脂肪族化合物(角质和软木脂)中与芳环相连的甲基 C(0 ~ 25 ppm)或聚亚甲基 C(25 ~ 50 ppm)
50 ~ 60	主要共振峰在 56 ppm 附近,为木质素来源的甲氧基 C 或含 N 烷基 C
60 ~ 90	主要共振峰在 73 ppm 附近,主要为碳水化合物(如纤维素、半纤维素和其他高分子碳水化合物及醇类)中的 C
90 ~ 110	共振峰在 105 ppm 附近,主要为多糖中的双氧烷基 C
110 ~ 145	共振峰主要在 115 ppm 和 130 ppm 处,为被羧基或羧甲基取代的芳香 C 以及 O、N 等取代基间位的连 H 芳香 C
145 ~ 160	主要共振峰在 150 ppm 附近,系木质素来源的被氧取代的酚基 C
160 ~ 190	共振峰主要在 174 ppm 附近,为羧酸及其衍生物(酯、酰胺、酸酐等)的羧基 C
190 ~ 220	为醛、酮、醌类化合物的羰基 C

蜈蚣草离体鲜根样品的<sup>13</sup>C-NMR波谱如图 1 所示,8 组不同处理的离体鲜根样品<sup>13</sup>C-NMR波谱基本一致,主要吸收峰位于 50 ~ 110 ppm 的烷氧基碳区中的 72 ppm 和 105 ppm 处.通过定性分析可知,在烷基碳区,主要为与芳环相连的甲基碳(21 ppm)和

无定形(CH<sub>2</sub>)<sub>n</sub>长链碳(30 ppm)<sup>[14,18-20]</sup>;在烷氧基碳区,主要为碳水化合物中 C2 至 C6 结构的吸收峰(63 ~ 64、71 ~ 74、82 ~ 89 ppm)和多糖中双氧烷基碳(乙缩醛)的吸收(105 ppm)<sup>[21-25]</sup>,其中 63 ~ 65 ppm 和 71 ~ 75 ppm 分别为碳水化合物中的伯醇和

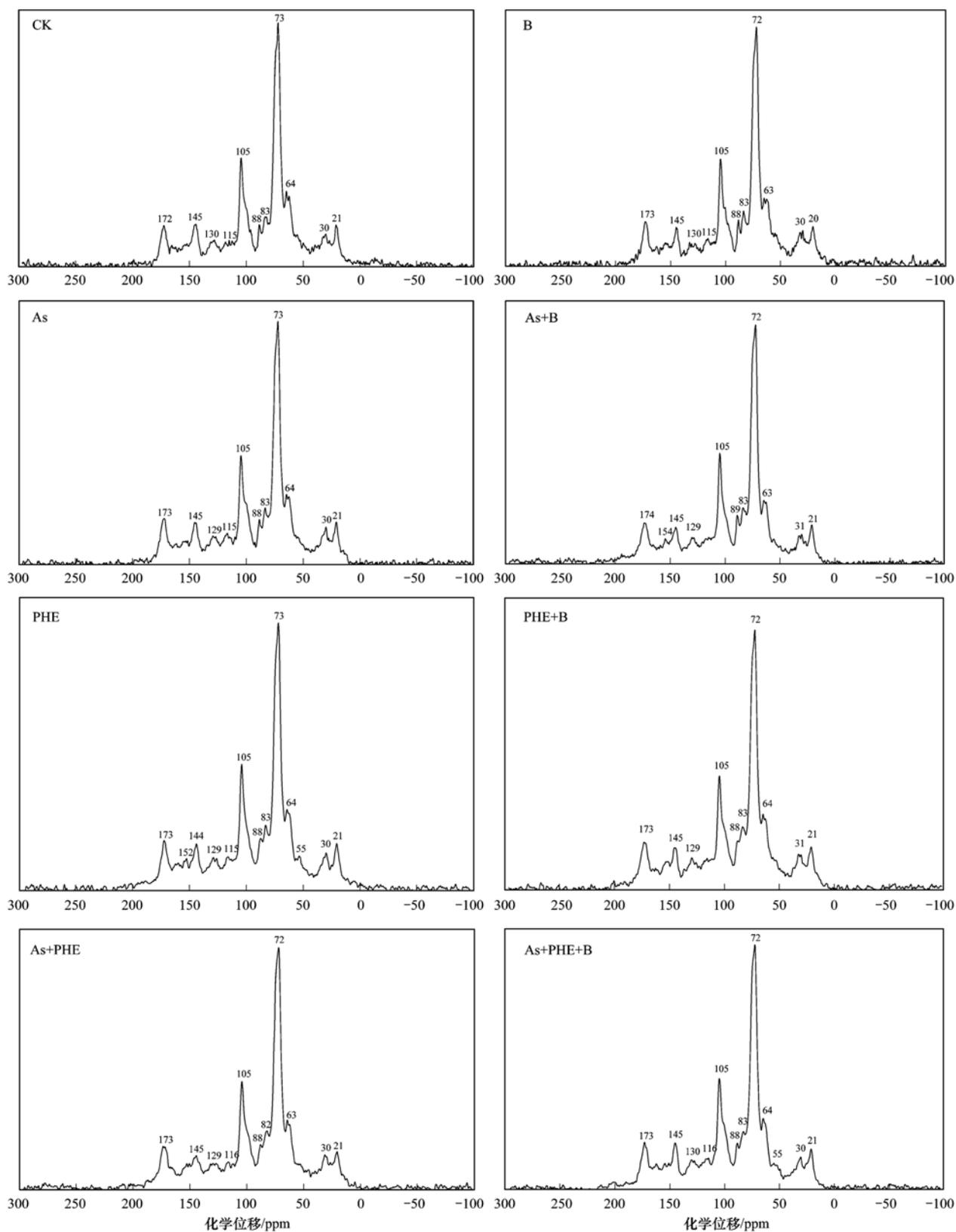


图 1 不同处理鲜根的 $^{13}\text{C}$ -NMR图谱

Fig. 1 Solid-state  $^{13}\text{C}$ -NMR spectra of the excised roots

仲醇基碳的吸收峰<sup>[26,27]</sup>,且主要来源于纤维素类物质的碳水化合物<sup>[28]</sup>;芳香碳区中,115 ppm和130

ppm的峰主要是被羧基或羧甲基取代的芳香碳以及与O、N等取代基间位的连H芳香碳的吸收

峰<sup>[14,26]</sup>, 而 145 ppm 为酚基碳的吸收峰. 羰基碳区的峰信号只在 173 ppm 处出现, 其他区域均没有峰信号出现, 表明其中无醛、酮、醌类化合物的羰基碳, 主要为羧酸、酯和酰胺类的羰基羧基碳<sup>[26,27,29]</sup>.

由表 4 可知, 蜈蚣草离体鲜根样品中的有机质以烷氧基碳比例最高 (65.51% ~ 70.58%), 其次为芳香碳 (13.36% ~ 18.41%) 和烷基碳 (10.21% ~ 12.65%), 羧基碳的比例最低 (3.41% ~ 5.78%). 与空白对照相比, 添加 As、PHE 后, 蜈蚣草鲜根样

品中的烷基碳和烷氧基碳比例降低, 芳香碳和羧基碳则分别增加了 29.4% ~ 37.8% 和 48.1% ~ 69.5%. 在实验处理组中, 菲处理后的蜈蚣草离体鲜根烷基碳和芳香碳比例最高, 而羧基碳比例最低; As + PHE 处理后的蜈蚣草离体鲜根中烷氧基碳比例最高, 其烷基碳比例则最低; 对于羧基碳而言, 比例最高的是 As + PHE + B 处理组. 在培养液中添加菲降解菌能够提高蜈蚣草离体鲜根中羧基碳比例, 但会降低鲜根中有机质的芳香性和疏水性.

表 4 鲜根样品中不同类型 C 的分布<sup>1)</sup>/%

Table 4 Carbon groups distribution in the excised roots of *P. vitatta* L./%

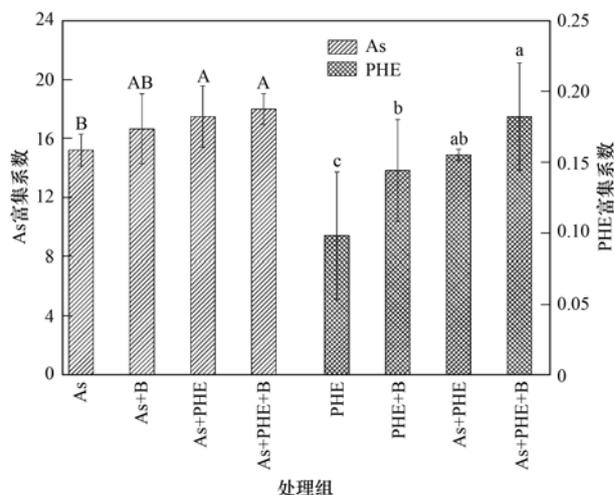
处理组	烷基 C	烷氧基 C	芳香 C	羧基 C	脂族 C	芳香性	疏水性
CK	12.65	70.58	13.36	3.41	83.23	13.83	35.15
B	10.89	65.51	18.35	5.25	76.40	19.37	41.32
As	10.41	66.36	17.94	5.28	76.77	18.94	39.57
PHE	10.93	65.61	18.41	5.05	76.54	19.39	41.52
As + B	10.40	66.90	17.29	5.41	77.30	18.28	38.29
PHE + B	10.22	66.54	17.95	5.29	76.76	18.95	39.22
As + PHE	10.21	67.07	17.30	5.42	77.28	18.29	37.95
As + PHE + B	10.22	66.61	17.39	5.78	76.83	18.46	38.14

1) 脂族 C = 烷基 C + 烷氧基 C; 芳香性 = 芳香 C × 100% / (脂族 C + 芳香 C); 疏水性 = (烷基 C + 芳香 C) × 100% / (烷氧基 C + 羧基 C)

### 3 讨论

#### 3.1 蜈蚣草根对砷与菲的吸收特征

蜈蚣草离体根能有效吸收环境中的砷和菲, 在砷、菲和菲降解菌存在条件下, 蜈蚣草离体根对砷、菲的吸收效果明显增强 (图 2): 加菲处理后根部砷富集能力提升, 砷富集系数为不加菲处理的一倍以上; 加砷处理后, 蜈蚣草离体鲜根中菲含量升高, 其富集系数分别是不加砷处理的 1.26 倍和 1.58 倍; 菲降解菌的添加对砷吸收略有促进作用, 但可使菲的富集有较大幅度提升. 推测导致这种积极作用的可能原因主要是污染物胁迫下蜈蚣草根分泌物组分和含量的改变促进了根部对砷和菲的吸收. 如多环芳烃胁迫会改变植物根系分泌的可溶性有机碳、草酸和多糖等组分和含量<sup>[30,31]</sup>, 蜈蚣草在砷胁迫下会产生大量的可溶性有机碳 (如植酸和草酸等有机酸) 根系分泌物<sup>[32]</sup>, 分泌的有机酸是能够促进植物对砷的吸收. 另外可能原因是微生物作用下改变了植物根对污染物的根表吸附, Gao 等<sup>[33]</sup> 研究发现过类似现象, 添加 PAHs 降解菌可以促进水稻根对菲和芘的根表吸附. Sun 等<sup>[5]</sup> 研究发现砷共存会影响蜈蚣草根对菲的吸收机制并推断砷可能改变了蜈蚣草根的细胞通透性或根构型, 从而影响了蜈蚣草根对菲的根表吸附.



不同字母表示各处理间的显著性差异,  $P < 0.05$

图 2 不同处理条件下蜈蚣草离体鲜根的富集系数

Fig. 2 BCF of arsenate and phenanthrene in the excised roots of *P. vitatta* L.

#### 3.2 蜈蚣草根富集菲和砷与碳基团的关系

采用固态<sup>13</sup>C-NMR技术对蜈蚣草离体鲜根中的主要碳基团进行研究发现, 离体鲜根样品的碳基团主要以烷氧基碳为主, 且主要来源于碳水化合物 (如纤维素和多糖等). 通过定性和定量分析表明, 砷和菲的添加会影响蜈蚣草离体鲜根的碳结构. 添加砷和菲后, 蜈蚣草离体鲜根样品的羧基碳比例增加, 且羧基碳的比例随着蜈蚣草体内砷、菲含量的

增加而升高。根据<sup>13</sup>C-NMR波谱特征以及碳化学位移归属可知,蜈蚣草离体鲜根样品中的羧基碳主要来自羧酸、酯和酰胺类物质。植物根部对有机污染物吸收方式可分主动吸收和被动吸收,非离子型的有机污染物主要是通过被动扩散的形式进入植物根系,这个过程也可简单看成是有机污染物在根部有机组分和环境介质之间的平衡分配过程。Gao等<sup>[34]</sup>研究了12种植物吸收积累PAHs的能力,发现根系富集系数与植物的脂肪含量呈显著正相关,高脂肪含量的植物根系对PAHs的富集、积累能力较强。Zhang等<sup>[35]</sup>研究发现,小球藻对菲的吸附容量均与极性官能团呈负相关关系,与烷基碳和聚亚甲基碳、芳香碳呈正相关关系,聚亚甲基碳、脂肪结构以及芳香结构对菲的吸附可同时起作用。

脂族碳与芳香碳的比值主要用以反映植物根中有机质分子结构的复杂程度,该比值越高表明蜈蚣草离体鲜根中芳香核结构越少,缩合程度越低,分子结构也越简单。添加砷和菲等外源物质后,蜈蚣草离体鲜根中有机质的芳香碳比例随着砷和菲含量的升高而增加,表明在砷、菲污染胁迫下,蜈蚣草离体鲜根为应对污染胁迫形成了分子结构更为稳定和复杂的芳香类有机质,从而提高离体鲜根的抗性和适应性。

#### 4 结论

蜈蚣草离体鲜根对砷和菲均表现出较强的吸收作用,砷的添加能促进蜈蚣草离体鲜根对菲的吸收,同样,菲也能提高砷的富集能力。菲降解菌的添加能够显著促进蜈蚣草离体鲜根对菲的吸收和富集作用,但对砷的吸收影响不大。采用固态<sup>13</sup>C-NMR技术研究发现,蜈蚣草离体鲜根中以烷氧碳为主,羧基碳比例最低,且主要来源于羧酸、酯和酰胺类物质。砷、菲和菲降解菌的添加会增加蜈蚣草离体鲜根中羧基碳的比例,提高芳香性和疏水性。

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