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蜈蚣草中芘与砷的赋存特征及其相互作用

张玉秀¹, 马旭^{1,2}, 廖晓勇^{2*}, 阎秀兰², 马栋², 龚雪刚²

(1. 中国矿业大学(北京)化学与环境工程学院, 环境生物工程系, 北京 100083; 2. 中国科学院地理科学与资源研究所, 环境损害与污染修复北京市重点实验室, 北京 100101)

摘要: 超富集植物蜈蚣草能够累积土壤环境中的砷, 为探明蜈蚣草植株中芘的赋存特征及其与砷的相互作用, 采用双光子激光共聚焦扫描显微技术检测观察芘在该植物中赋存和分布规律。结果表明, 添加芘导致蜈蚣草各部位砷含量显著降低, 其中叶部和茎部砷含量下降幅度达35%左右, 根部砷含量减少20.5%。芘的添加使得植物各部位三价砷比例下降, 五价砷比例升高, 蜈蚣草叶部三价砷和五价砷赋存浓度下降幅度最大, 下降幅度分别为42.2%和32.49%。添加砷可促进蜈蚣草根部和茎部中芘的积累, 每株分别增加了9.8 μg 和139 ng, 但对叶部中芘的积累无显著影响。芘主要分布于蜈蚣草细胞膜与细胞内部的细胞器膜结构及细胞核核膜附近, 但细胞质中芘的含量较少, 茎部韧皮部与皮层以及叶部栅栏组织和海绵组织中几乎没有检测到芘。

关键词: 芘; 砷; 蜈蚣草; 植物修复; 交互影响

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Occurrence Characteristics of Pyrene and Arsenate and Their Interaction in *Pteris vittata* L.

ZHANG Yu-xiu¹, MA Xu^{1,2}, LIAO Xiao-yong^{2*}, YAN Xiu-lan², MA Dong², GONG Xue-gang²

(1. Department of Environmental and Biological Engineering, School of Chemical and Environmental Engineering, China University of Mining and Technology (Beijing), Beijing 100083, China; 2. Beijing Key Laboratory of Environmental Damage Assessment and Remediation, Institute of Geographical Sciences and Natural Resources Research, Chinese Academy of Sciences, Beijing 100101, China)

Abstract: *Pteris vittata* L. can absorb and accumulate high arsenic levels in soil. To clarify the occurrence characteristics of pyrene (PYR) and arsenate(As) as well as their interaction in *P. vittata* L., the hosting and distribution rules of PYR were determined via two-photon laser scanning confocal microscopy (TPLSCM). The results showed that PYR addition resulted in obviously lower concentrations of total As in various parts of *P. vittata*, with a largest decrease of about 35% in the leaves and stem, and 20.5% in the roots. PYR addition could also decrease the proportion of trivalent arsenic and increased that of pentavalent arsenate in different parts of *P. vittata*. The concentrations of trivalent and pentavalent arsenic in the leaves of *P. vittata* showed the largest decrement, which were 42.2% and 32.49%, respectively. Arsenate addition increased the accumulation of PYR in the root and stem of *P. vittata* by 9.8 μg and 139 ng per plant, respectively, while no obvious influence was found on the PYR in the leaves. Pyrene mainly attached to the cell membrane and other membrane structure such as nuclear membrane and organelle membrane, and there was less pyrene in the cytoplasm. There was little PYR in the phloem and cortex in the stem as well as palisade tissue and spongy tissue in leaves.

Key words: pyrene; arsenate; *Pteris vittata* L.; phytoremediation; interaction

多环芳烃(polycyclic aromatic hydrocarbons, PAHs)和砷是环境中普遍存在的有毒污染物,是我国第一批公布的68种优先控制污染物,同样被美国环保署列为优先级污染物,具有高毒性、致癌、致畸作用^[1]。这两种污染物都会出现在木材生产厂^[2]、燃气制造厂^[3]、焦电厂^[4]、采矿与冶金^[5]等工业企业场地及周边区域,它们的复合污染及危害问题已引起广泛关注^[6,7]。

砷超富集植物蜈蚣草处于高砷环境中不被损伤且可从砷污染土壤中高量累积砷,并将砷区隔在羽叶部分的液泡中^[8]。砷在环境中主要的存在形式是As(V),由于其化学结构类似于磷,它主要通过磷

的运输通道吸收运输^[9]。蜈蚣草通过As(V)还原酶将As(V)还原为As(III),这个过程中三价砷会与富硫醇基团的多肽类络合形成植物螯合态砷,并储存隔离在液泡中^[10]。

PAHs作为一类非极性亲脂性有机化合物^[11],由于其低水溶性特点导致其在环境中能够持久存在^[12,13],具有低水溶性、低亨利定律常数、高 K_{ow}

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作者简介: 张玉秀(1962~),女,博士,教授,主要研究方向为矿区环境与污染土壤修复, E-mail: zhangyuxiu@cumtb.edu.cn

* 通讯联系人, E-mail: liaoxy@igsnrr.ac.cn

(octanol-water partition coefficients) 的物理化学性质,能够通过植物的根系吸收或是叶片的蜡质层进入植物体内,并最终进入食物链,影响人类健康. 芘(pyrene)是多环芳烃的一种,由 4 个苯环线性组合而成. 芘是中环多环芳烃的代表,辛醇水分配系数($\lg K_{ow} = 5.22$)高,水溶解度($S = 0.135 \text{ mg} \cdot \text{L}^{-1}$)低,但易溶于乙醇、二硫化碳、乙醚、苯和甲苯等有机溶剂.

本课题组前期研究发现,蜈蚣草能够耐受砷和多环芳烃^[14],且砷、菲共存显著影响蜈蚣草体内砷的赋存形态,添加砷可以改变蜈蚣草根对菲的吸收,显著提高蜈蚣草地下部菲含量^[15]. 本研究以 PAHs 中环中最具有代表性的芘为目标物,探明砷-芘在蜈蚣草植株体内交互吸收与赋存的规律. 并采用双光子激光共聚焦扫描显微镜技术观察芘在蜈蚣草在组织细胞中分布和贮存信号,揭示其在细胞组织中的赋存规律,进一步解释 PAHs 和砷在超富集植物蜈蚣草中的生物化学行为和交互机制.

1 材料与方法

1.1 实验设计

将来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($\text{pH} = 6.0$)中进行培养. 4~5 个月后,选取长势优良且相似的蜈蚣草苗备用.

实验共设计 4 组处理:空白对照、单独砷、芘、芘加砷. 实验中砷选取砷酸钠,五价砷的配置浓度为 $10 \text{ mg} \cdot \text{L}^{-1}$,将芘单独溶解于丙酮有机试剂中,并添加到水培营养液中,使得芘浓度为 $2 \text{ mg} \cdot \text{L}^{-1}$. 每 3 d 换一次营养液,4 个周期共 12 d 后将蜈蚣草收获. 将收获的蜈蚣草用清水洗净,自然晾干,将叶、茎、根部分开. 选取一部分蜈蚣草 105℃ 杀青 30 min, 65℃ 烘干 24 h,用于蜈蚣草总砷测定. 剩余部分蜈蚣草冷冻干燥 24 h,称量干重,冰浴磨碎 -80℃ 冷藏,用于砷形态和芘的测定.

1.2 砷形态与总砷

将烘干的植物样品采用 $\text{HNO}_3\text{-HClO}_4$ (US EPA 3050) 方法消煮,定容稀释,原子荧光光度计 (AFS-9130) 测定植株各个部分总砷含量. 将冰浴磨碎后的蜈蚣草,用甲醇水(体积比 1:1)超声萃取 3 次,每次 30 min,每 15 min 振荡一次. 将萃取液离心去除

植物碎屑,萃取液旋转蒸发去除甲醇后,定容稀释,应用双道原子荧光光度计 (AFS-LC-9130) 测定砷形态^[15]. 用 IC Anion PW 阴离子交换色谱分离三价砷 As(III),五价砷 As(V),亚甲基砷酸盐 MMA 和甲基砷酸盐 DMA,在 $\text{pH} = 6.0$ 条件下,用 $15 \text{ mmol} \cdot \text{L}^{-1}$ $(\text{NH}_4)_2\text{HPO}_4$ 作为流动相,流速为 $1.0 \text{ mL} \cdot \text{min}^{-1}$. 使用 2% KBH_4 和 0.5% KOH 为还原剂,7% HCl 为载流. 高电倍增管负高压为 290 V、灯电流为 100 mA、载气流速为 $300 \text{ mL} \cdot \text{min}^{-1}$,屏蔽气流速为 $600 \text{ mL} \cdot \text{min}^{-1}$. 分析过程所用试剂均为优级纯,采用国家标准参比物质 (GBW-07603) 作为分析质量控制的标准,标样测定结果均在参比物质允许误差范围之内. 叶、茎、根部砷形态的提取效率分别为 80.5% ~ 83.8%、79.9% ~ 80.2%、66.3% ~ 77.9%.

1.3 芘的测定

将冰浴磨碎后的植物样品用丙酮和二氯甲烷混合溶液(1:1,体积比)超声萃取 3 次,每次 30 min,每 15 min 振荡一次. 将萃取液离心去除植物碎屑,取上层澄清液旋转蒸发浓缩至 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,承接溶液旋蒸至约 1 mL,加入 7~8 mL 正己烷,再次旋蒸至约 0.5 mL,加内标、定容至 1 mL,利用 GC-MS (Agilent6890) 测定含量, ($n = 4$, $\text{RSD} < 10.5$),回收率 92% ~ 98%.

1.4 TPCLSM 技术观测蜈蚣草样品中 PAHs 信号

将蜈蚣草样品用蒸馏水洗净,制作活体植物切片,中性树胶封片,应用双光子激光共聚焦扫描显微镜 (Carl Zeiss LSM 780) 观测. 物镜为 W plan-Apochromat $20 \times / 1.0 \text{ DIC}(\text{UV}) \text{ VIS-IR M27 } 75 \text{ mm}$,设置激发波长为 710 nm,发射波长为 740 nm,检测波长为 371~495 nm. 并定义红色为芘伪彩色信号,绿色为植物自身荧光信号.

1.5 数据与图像处理

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 8.0 软件绘制图表;显微图片采用 ZEN 2012 blue edition 显微图像分析软件处理.

2 结果与分析

2.1 蜈蚣草中砷与芘的含量

表 1 为添加芘与砷处理下,蜈蚣草根、茎、叶部砷和芘的含量. 添加芘使得蜈蚣草各个部位砷含量均有不同程度的下降. 较未加芘处理,添加芘处理中叶部和茎部总砷下降幅度最大,达到 35% 左

右, 浓度分别下降了 113.2 $\text{mg}\cdot\text{kg}^{-1}$ 、71.35 $\text{mg}\cdot\text{kg}^{-1}$. 而砷对根部砷影响相对较小, 砷的浓度下降了 20.5%.

蜈蚣草可通过根部吸收砷并运输至地上部位, 根部对砷的吸收量(以 DW 计)最大, 能够达到 2.27 $\text{mg}\cdot\text{kg}^{-1}$, 叶部与茎部砷的含量为 420 ~ 586

$\mu\text{g}\cdot\text{kg}^{-1}$, 是根部砷含量的 18.5% ~ 25.8%. 添加砷对蜈蚣草吸收砷有显著影响, 提高了茎部与根部砷的赋存含量, 而降低了叶部砷的含量. 与只加砷相比, 添加砷后叶部砷的含量减少了 21.5%, 而添加砷后, 根部砷的含量增加了 4.032 $\text{mg}\cdot\text{kg}^{-1}$, 提升了 1.77 倍, 茎部砷含量提高了 36.2%.

表 1 蜈蚣草各部分总砷和砷的含量¹⁾

处理组	叶		茎		根	
	总砷/ $\text{mg}\cdot\text{kg}^{-1}$	砷/ $\mu\text{g}\cdot\text{kg}^{-1}$	总砷/ $\text{mg}\cdot\text{kg}^{-1}$	砷/ $\mu\text{g}\cdot\text{kg}^{-1}$	总砷/ $\text{mg}\cdot\text{kg}^{-1}$	砷/ $\mu\text{g}\cdot\text{kg}^{-1}$
CK	0.087 ± 0.04c	—	0.053 ± 0.01c	—	0.017 ± 0.006c	—
As	315 ± 14.8a	—	207 ± 18.78a	—	115 ± 17.5a	—
PYR	0.033 ± 0.02c	586 ± 32.9A	0.05 ± 0.01c	420 ± 46.4B	0.04 ± 0.01c	2 272 ± 137B
PYR + As	202 ± 13.8b	460 ± 52.4B	136 ± 7.65b	552 ± 55.8A	91.4 ± 6.36b	6 304 ± 396A

1) “—”表示忽略不计或未检出; 大、小写字母代表列内各处理间的显著性差异($P < 0.05$), 相同字母为无显著性差异, 相反为达到显著性差异

2.2 砷对蜈蚣草中砷赋存形态的影响

图 1 为蜈蚣草各部分三价砷和五价砷的含量与比例分布. 与未添加砷处理相比, 添加砷均使得叶、茎、根这 3 部分中三价砷和五价砷含量显著降低. 其中叶部三价砷与五价砷下降幅度最大, 分别下降了 42.2% 和 32.49%, 而茎部和根部中三价砷和五价砷含量降低了 36.8% ~ 37.6% 和 26.2% ~ 29.9%. 各部分中三价砷占总砷的比例略有下降, 五价砷占总砷的比例略有升高, 说明砷的添加可能对于蜈蚣草各部五价砷的还原能力有较小的抑制作用.

2.3 蜈蚣草中砷和砷的累积量

砷累积量呈现出叶部 > 根部 > 茎部的规律(图 2), 且砷对于蜈蚣草砷累积量的抑制作用明显, 与未加砷处理, 添加砷导致砷处理的植株叶部砷累积量下降了 162.4 $\mu\text{g}\cdot\text{株}^{-1}$, 降低幅度为 24.0%, 茎部

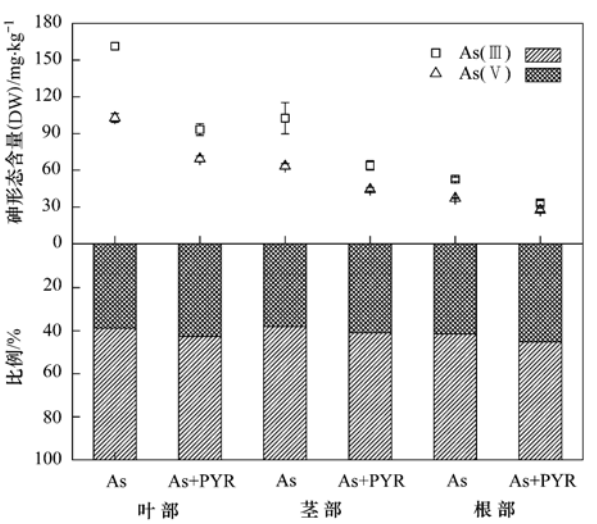


图 1 蜈蚣草中三价砷和五价砷含量与比例

Fig. 1 Concentrations and proportions of As(Ⅲ) and As(Ⅴ)

与根部砷累积量分别下降了 71.5 $\mu\text{g}\cdot\text{株}^{-1}$ 、63.2

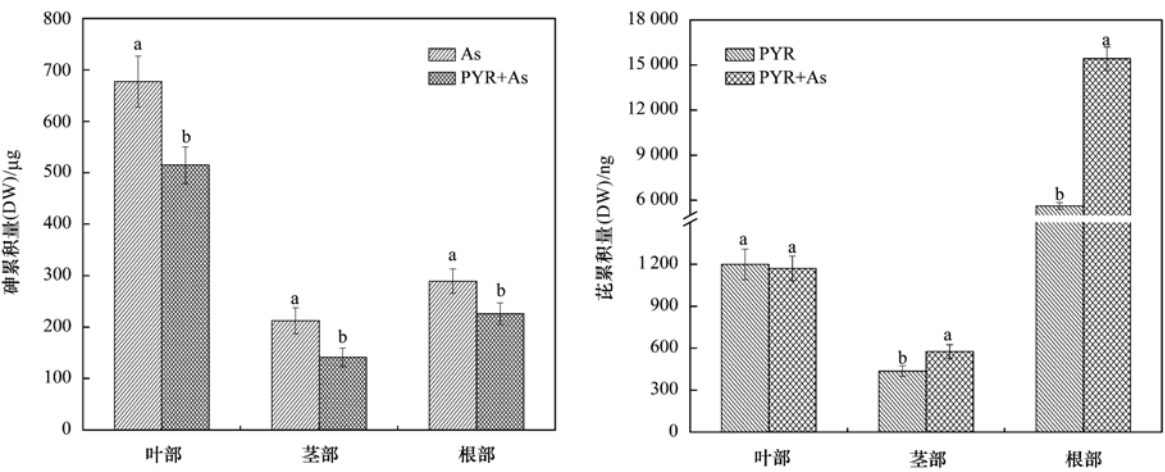


图 2 蜈蚣草中砷累积量和砷累积量

Fig. 2 Arsenic accumulation and pyrene accumulation in *P. vittata* L.

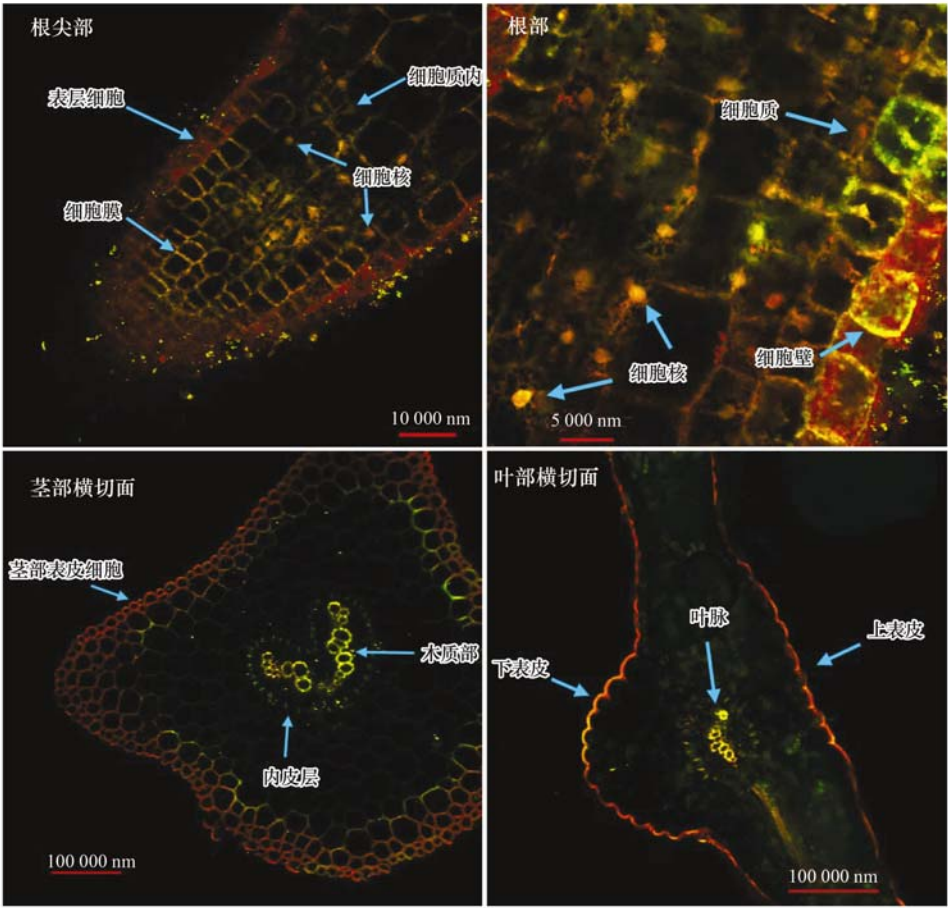
$\mu\text{g}\cdot\text{株}^{-1}$,其中茎部的下降幅度最大,为 33.6%.

蜈蚣草根对茈积累量最大,能够达到 $5.6\mu\text{g}\cdot\text{株}^{-1}$,而叶部与茎部茈积累量分别为 $1\,200\text{ ng}\cdot\text{株}^{-1}$ 、 $436\text{ ng}\cdot\text{株}^{-1}$ (以 DW 计). 添加砷对于蜈蚣草茎部与根部茈积累量有显著影响,能够提高蜈蚣草植株中茈的积累量,而对于叶部茈积累量影响不大. 与未加砷相比,添加砷处理蜈蚣草根茈积累量能够达到 $15.4\mu\text{g}\cdot\text{株}^{-1}$,而根部茈积累量增加 $9.8\mu\text{g}\cdot\text{株}^{-1}$.

2.4 蜈蚣草组织中茈的微区分布

图 3 为采用双光子激光共聚焦显微镜观察蜈蚣草组织细胞中茈的原位信号分布. 在蜈蚣草根尖部位,茈主要附着于根部的表皮细胞. 在表皮细胞中,细胞质、细胞膜与细胞核中茈的含量均很高. 而在根

部内部细胞中,茈较多地赋存于细胞的细胞膜、细胞核中,细胞质中茈的信号较弱,含量较少(图 3 根尖部、根部). 在茎部,茈主要分布于 U 型维管束(vascular bundle)的内皮层(endodermis)和木质部(xylem)细胞的细胞膜以及茎部外缘表皮细胞(epidermis)的细胞膜中,其中茈信号的强弱关系为:木质部细胞的细胞膜>茎部外缘表皮细胞>内皮层细胞,而在木质部与内皮层之间的韧皮部(phloem)和内皮层与外皮层之间的皮层(cortex)形成了茈的空白赋存区域(图 3 茎部横切面). 在蜈蚣草羽叶部位,茈主要赋存于上下表皮细胞、叶脉组织当中,栅栏组织和海绵组织中几乎没有茈的赋存(图 3 叶部横切面). 这种特征类似于茎部茈的赋存特征,蜈蚣草植株的这种特征对于减轻茈对植株的伤害有很大的作用.



红色为茈荧光信号,绿色为植物本身荧光信号,蓝色箭头指向茈信号贮存位置

图 3 蜈蚣草茈信号的微观分布示意

Fig. 3 Microscopic distribution of pyrene signals in *Pteris vittata* L.

3 讨论

3.1 砷与茈在蜈蚣草体内吸收与赋存及交互影响

蜈蚣草中砷能够促进根部、茎部茈的积累,而茈抑制砷的吸收与贮存. 加砷处理后,除叶部茈的

含量降低外,添加砷均促进了茈在根部和茎部的吸收与赋存,其中根部茈的赋存含量增长了 1.77 倍,茎部茈含量提高了 36.2%. 根部茈的含量远远高于地上部分,添加砷导致茈的转运系数显著下降(表 2). 关于砷为何能够提升茈在蜈蚣草根部的赋存浓

度, Sun 等^[15]在研究菲与砷在蜈蚣草体内交互作用过程中得出砷能够提升根部菲的含量, 并推断有可能是砷影响了菲在蜈蚣草的根表吸收能力以及其在蜈蚣草根内的降解过程, 进而导致了菲在蜈蚣草地部的累积, 或有可能砷存在下改变了蜈蚣草根的细胞通透性或者根构型, 影响了蜈蚣草根对菲的根表吸附。

表 2 蜈蚣草砷的转移系数

Table 2 Transfer factor of pyrene in *P. vitatta* L.

项目	砷		砷	
处理条件	PYR	PYR + As	PYR	PYR + As
转运系数 TF	2.44	2.53	0.235	0.077 6

砷的添加影响了蜈蚣草植株对五价砷的吸收、赋存以及在蜈蚣草体内的赋存形态。加砷使得蜈蚣草各个部位总砷含量有不同程度的下降, 下降幅度为 20.5% ~ 35.9%。且添加砷使得蜈蚣草不同部位五价砷与三价砷含量均减少, 较小地抑制了五价砷的还原转化。Sun 等^[15]在研究菲和砷在蜈蚣草的交互行为时得到, 菲会抑制蜈蚣草根对砷的吸收和转运, 此外 Lin 等^[16]在研究玉米 (*Zea mays* L.) 对砷和铜的交互作用时得出, 砷的添加能够降低玉米对铜的富集; Wang 等^[17]在研究东南景天 (*Sedum alfredii*) 对镉和砷复合污染土壤修复实验中也发现, 砷的添加降低了景天属地上部分镉的浓度, 进而降低了对镉的富集效率。

PAHs 不仅影响蜈蚣草植株对砷的吸收, 并且还会影响砷的赋存状态, 砷的添加会抑制蜈蚣草各部五价砷向三价砷的转化, 蜈蚣草中三价砷占总砷的比例略有下降, 五价砷占总砷的比例略有升高, 这说明砷的添加可能对于蜈蚣草各部五价砷的还原能力有较小的抑制作用。砷可以与细胞质中的悬浮物质、蛋白质发生交互反应, 并产生生物细胞毒性^[18], Sun 等^[15]提出菲的添加抑制蜈蚣草对五价砷的还原能力, 使得地上部三价砷显著降低。由此推断砷可能与细胞中五价砷的转运蛋白及砷还原酶等蛋白质发生交互反应, 使得细胞损伤或是转运蛋白及还原酶中毒, 影响五价砷的正常吸收与转化, 进而影响五价砷在植株中的含量与赋存形态。

3.2 砷在蜈蚣草植株内分布与赋存规律

通过双光子激光共聚焦显微技术原位观测组织细胞中砷的赋存位点, 砷在蜈蚣草植株中的赋存规律为: 在蜈蚣草微观组织细胞中, 细胞外环境中的砷首先附着于细胞膜外层, 并与细胞膜内层结合, 通过跨膜运输进入到细胞内部, 主要附着于细胞内部的

细胞器膜结构与细胞核核膜附近, 少量砷结合细胞质中有机化合物并储存于细胞质中。在蜈蚣草茎部韧皮部与皮层两个部位以及叶部栅栏组织和海绵组织中几乎没有砷的赋存, 这有助于降低砷对蜈蚣草本身的毒害作用。

植物能够通过不同的途径吸收 PAHs, 一方面植物的叶子可以通过表面蜡质吸收沉积在上面富含 PAHs 的固体颗粒物中的 PAHs^[19~21], 也可以通过根部根系吸收 PAHs 并转运到地上部的茎部和叶部^[22~25]。植物吸收与赋存 PAHs 的主要动力来自于植株的蒸腾作用与化学浓度梯度^[26~29]。PAHs 高 K_{ow} (辛醇/水分布系数) 容易进入有机相^[30], 并且细胞膜磷脂双分子层外部亲水性, 内部亲脂性的独特特点使得砷在细胞膜的吸附量较大^[26, 31], 化合物的亲油性是植物吸收量的决定性因素^[27]。砷作为 PAHs 中的一种, 具有低水溶性、低亨利定律常数、高 K_{ow} (octanol-water partition coefficients) ($\lg K_{ow} = 5.22$) 的物理化学性质, 能够与细胞膜和大分子有机物质等亲脂性结构亲和并附着。Kang 等^[26]在研究黑麦草根细胞中砷的亚细胞动力学规律时发现, 砷表现出很强的吸收性和难以新陈代谢的特性, 首先吸附在根的细胞壁上, 然后透过细胞膜进入到细胞内部的一些细胞器里面。而砷在亚细胞的分布与各个细胞器中脂含量成正相关性, 其高亲脂性使得在细胞器膜的吸附量较大。Ren 等^[32]也证实菲和蒽可以通过大肠杆菌的细胞膜的磷脂质进入到细胞质, 且菲与大肠杆菌细胞质中的悬浮物质、有机质能够发生交互反应。

4 结论

(1) 砷和砷在蜈蚣草植株中交互作用显著, 砷的添加能够促进蜈蚣草根部和茎部砷的吸收和赋存, 降低了叶部砷的含量。

(2) 砷抑制蜈蚣草各部位对五价砷的吸收和转化, 且植株体内三价砷的含量与比例均下降, 五价砷含量下降, 而比例上升。

(3) 通过双光子激光共聚焦显微镜技术得出砷在蜈蚣草组织细胞中的赋存与分布规律: 在蜈蚣草组织细胞中, 砷主要附着于细胞膜附近, 并依附于细胞内部的细胞器膜结构与细胞核核膜。在蜈蚣草茎部韧皮部与皮层两个部位以及叶部栅栏组织和海绵组织中几乎没有砷的赋存, 该赋存特征有助于降低砷对蜈蚣草组织细胞的毒害作用。

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