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泉州湾湿地生物对环境污染物的毒理学响应特性研究

——桐花树、凤眼莲、可口革囊星虫

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TOXICOLOGICAL RESPONSE OF WETLAND ORGANISMS TO ENVIRONMENTAL POLLUTION IN QUANZHOU BAY, FUJIAN PROVINCE, CHINA

——*Aegiceras corniculatum, Eichhornia crassipes, Phascolosoma esculenta*

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内 容 摘 要

本文以泉州湾湿地生物桐花树（*Aegiceras corniculatum*）、凤眼莲（*Eichhornia crassipes*）、可口革囊星虫(*Phscolosoma esculenta*)为研究对象，从环境污染物在湿地系统的分布特点、赋存形态以及生理生态和分子毒理学水平探讨泉州湾湿地生物对环境污染物的毒理学响应特性。得出的主要结论是：

1 泉州湾常见植物的生境土壤受中度以上的 Zn、Pb 污染，且 Zn 与 Pb 污染呈显著正相关关系。在 Cu、Zn、Pb 各重金属形态中，交换态和碳酸盐结合态仅占较小比例,而还原态占较大比例， Cu 和 Zn 的氧化态含量较高,而 Pb 的氧化态含量较低。Cu、Zn、Pb 在桐花树群落和补血草群落样地的沉积物里具相同的形态分布模式，芦苇和空心莲子草群落样地沉积物具有相同的 Cu、Zn、Pb 形态分布模式。大多数重金属分布于非流动相而不易被生物使用。与桐花树、补血草和芦苇相比，空心莲子草对 Cu、Zn、Pb 具有最好的累积性能。芦苇对 Zn、Pb 有较好的耐受性和转移性能。泉州湾海岸植物栖息土壤中 K 含量较高。相对而言，空心莲子草、莲子草、龙葵、芦苇对 Cu、Zn、Pb 耐性高、累积能力强，属于有环境修复潜力的植物，可作为对重金属污染环境的绿色修复材料考虑。桐花树属于抗重金属性能很强的物种，不属于敏感指示物种。藜的根、茎、叶中具高含量的 K 元素，对土壤中的 K 具有较高的吸收和迁移能力。落葵、莲子草、芦苇、藜的叶中 N 含量较高，落葵和莲子草叶则表现低 P 含量，高 N/P 比值。本试验研究的泉州湾自然生境若干海岸植物对 Cu、Zn、Pb 的累积与其 N、P、K 营养元素含量之间未表现显著的相关性。

2 桐花树幼苗的碳代谢受 Pb 胁迫源于时间和浓度的双重影响，和第 3 d 的胁迫处理相比，15 d Pb 胁迫下的 Pb 胁迫幼苗叶淀粉酶活性总体高于 3 d Pb 胁迫，但两者淀粉酶活性变化趋势相似，在 Pb^{2+}浓度不超过 2000 mg • kg^{-1} 时，随着 Pb^{2+}浓度的增加而逐渐升高，并在 Pb^{2+}浓度为 2000 mg • kg^{-1} 时达到最大值，随后，当 Pb^{2+}浓度大于 2000 mg • kg^{-1} 时，淀粉酶活性则急剧下降。当在 Pb^{2+}浓度大于 500 mg • kg^{-1} 时，对叶绿素的合成表现出一定的破坏作用，且对叶绿素 a 的破坏作用较叶绿素 b 大。

3 Pb^{2+}胁迫使桐花树幼苗根、茎、叶的氮代谢受不同程度影响。0.1～2 mmol·L^{-1} Pb^{2+}胁迫使营养液中培养的桐花树幼苗根、茎、叶 NADH-GOGAT 的活性降低，丙二醛含量增加，使根和茎的 GS 活性降低，而叶 GS 在 0.1～1 mmol·$L^{-1}$$Pb^{2+}$胁迫下活性增强高于对照，但受 2 mmol·$L^{-1}$ 较高浓度 Pb^{2+}抑制。无论是否有 Pb^{2+}胁迫，叶的 GS 活性都高于茎和根，而根的 NADH-GOGAT 活性始终高于茎和叶。

根的 GS 和 NADH-GOGAT 活性受 Pb^{2+}胁迫抑制的程度明显大于茎、叶，Pb^{2+}对根的初级氮代谢影响最大。 MDA 含量与 GS 和 NADH-GOGAT 活性呈负相关关系。

4　在桐花树根、茎、叶三器官中以幼叶的 SOD 酶活最大，根的 SOD 活性弱于茎叶；在自然生境中生长的桐花树根茎叶 SOD 同工酶没有特异性差异，主要以 Cu·Zn–SOD 为主，其同工酶区带与其活性表现结果是相一致的，即根的 SOD 活力显现较弱，茎叶 SOD 活力表现较强。

5　CTAB 法提取红树植物桐花树总 RNA 是理想的方法；分离克隆所获得桐花树幼茎 Cu,Zn-SOD 基因片段 278 个碱基，该碱基序列在 NCBI 基因库中的登录号为 DQ913822。桐花树的 Cu,Zn-SOD 序列是 Cu,Zn-SOD 阅读框的部分序列(靠近 3’端，即 C 端)，从其蛋白质序列来看，与陆地棉（*Gossypium hirsutum*）等其它植物的同源性达 93%。

6　桐花树幼苗茎尖组织可通过增强 SOD 酶活性对一定浓度范围的 Cd^{2+}、Pb^{2+}胁迫体现出保护性的适应反应。Cu,Zn-SOD 基因对 Pb^{2+}胁迫有较好的耐受性，而敏感于 Cd^{2+}胁迫，40 μmol•L^{-1} Cd^{2+}胁迫培养使桐花树幼苗茎尖 Cu,Zn-SOD 基因表达量极显著上调。Cd^{2+}、Pb^{2+}对桐花树幼苗茎尖 SOD 代谢体现出拮抗作用。

7　分离克隆泉州湾桐花树幼苗幼茎 PM H^{+}-ATPase 基因片段 323 bp。桐花树幼苗根茎叶 PM H^{+}-ATPase 活性由强到弱依次为：茎 > 根 > 叶。叶 PM H^{+}-ATPase 基因表达明显高于根茎，茎和根 PM H^{+}-ATPase 基因表达没有显著差异。Cd^{2+}、Pb^{2+}胁迫使叶的 PM H^{+}-ATPase 基因表达上调，使根的 PM H^{+}-ATPase 基因表达下调，高浓度 Cd^{2+}胁迫使幼茎 PM H^{+}-ATPase 基因表达急剧上调，Pb^{2+}的加入会减弱其对 Cd^{2+}的敏感性。

8　0.1 mmol·L^{-1}～2 mmol·$L^{-1}$$Pb^{2+}$胁迫 30 d 的凤眼莲叶片中叶绿素含量显著升高，但叶绿素 a/b 值显著降低，低浓度 Pb^{2+}胁迫会诱导 Hill 反应活性升高，较高浓度 Pb^{2+}胁迫(≥ 0.5 mmol·L^{-1} Pb^{2+})使凤眼莲叶片中 Hill 反应活性随着 Pb^{2+}胁迫浓度的增加越来越低，显著低于对照。高浓度 Pb^{2+}胁迫导致部分叶绿体结构受损，膜系统被破坏，内囊体破裂，基粒致密性降低。低强度 Pb^{2+}胁迫可以激发组织内抗氧化保护系统的作用使活性氧的产生与清除之间建立了新的平衡，使丙二醛含量降低，但较强 Pb^{2+}胁迫(≥ 1 mmol·L^{-1} Pb^{2+})使膜脂质过氧化程度显著加重。

9　铅污染影响凤眼莲根系的细菌、放线菌、霉菌和酵母菌等的活菌数和相应水样的 DO（溶解氧量）、COD（化学需氧量）。Pb^{2+}(0 mmol·L^{-1}、0.1 mmol·L^{-1}、

0.25 mmol·L^{-1}、0.5 mmol·L^{-1}、1.0 mmol·L^{-1}、2.0 mmol·L^{-1}) 胁迫 27 d 后水体中各微生物的数量较胁迫 3d 后的要少，且细菌、放线菌、霉菌和酵母菌等的数量均呈现先增加后减少的变化规律，其中细菌为凤眼莲根系的主导菌群，其对 Pb^{2+} 的耐受浓度为 0.25 mmol·L^{-1}，而真菌对 Pb^{2+}的耐受浓度为 0.50 mmol·L^{-1}。各水样在不同时间里 DO 值有减小的趋势，COD 值表现为增大的趋势，但同一时间里 DO 值随 Pb 浓度的增加先增加后减少，COD 值则是先减少后增加, DO 值和 COD 值与凤眼莲根系微生物的生长存在着密切的内在联系。

10 Cd^{2+} 对可口革囊星虫 96 h 的 LC_{50} 为 35.84 μg·g^{-1}。与对照组相比，较低 Cd^{2+} 浓度处理组肠 SOD 和 CAT 活性有显著升高;而浓度较高处理组没有显著变化。体液 SOD 活性在 Cd^{2+}浓度为 2 μmol·L^{-1} 时显著下降，在较高浓度时与对照组水平无显著差异; CAT 在较低 Cd^{2+} 浓度时有显著上升，在 0.512 mmol·L^{-1} 时显著下降。与对照相比，MDA 在较低 Cd^{2+} 浓度处理时，有较大变化，较高 Cd^{2+} 浓度处理时亦无显著变化。

11 100～10000 ng·g^{-1}BaP 胁迫使星虫体壁的 BaP 含量均明显升高，但星虫对 BaP 胁迫具有很强的抗性，累积性不强。分离克隆并鉴定了可口革囊星虫体壁 411bp Mn-SOD(在 NCBI 基因库中的登录号为 EF062359)和 315 bp 18srRNA 的两个基因片段。所克隆的Mn-SOD与部分虾蟹鱼的Mn-SOD同源性较高，经NCBI在线氨基酸比对同源性达 68％以上；18srRNA 基因片段与星虫纲的多种星虫 18 srRNA 的同源性达到 100％。

12 100～10000 ng·g^{-1} 浓度范围的 BaP 胁迫对可口革囊星虫体壁总 SOD 与 Mn-SOD 活性均有影响，SOD 活性随 BaP 胁迫浓度的变化没有一定的规律性，但随胁迫时间的变化趋势较为一致。各胁迫组的 SOD 活性都在胁迫的第 4 d 达到最高值，之后随时间的延长，活性逐渐降低。随着胁迫时间的延长，Mn-SOD 活性在总 SOD 活性中的比重明显增大。Mn-SOD 基因敏感于 BaP 胁迫，胁迫 4 h 内 Mn-SOD 基因表达迅速上调到峰值，随后随胁迫时间的延长，上调幅度降低，到胁迫 2d 时走向下调趋势。Mn-SOD 生理活性上的响应迟滞于 Mn-SOD 基因的表达响应。从污染物的生物标志物应用方面考虑，SOD 的分子响应比生理响应更容易预警环境的早期污染。

关键词：泉州湾湿地， 桐花树， 凤眼莲， 可口革囊星虫， 毒理学响应， 化学污染物, Cu,Zn-SOD， Mn-SOD， PM H^{+}-ATPase

Abstract

The toxicological response characteristic of the wetland organisms including *Aegiceras corniculatum, Eichhornia crassipes, Phascolosoma esculenta* in Quanzhou Bay to environmental pollution were researched based on pollution distribution and their chemical partition and physiological and ecological and molecular toxicological level. The main results are as follows:

1 The sediment soils inhabited by plants of the Quanzhou Bay are moderately or strongly contaminated with Zn and Pb, and the concentrations of Pb and Zn are correlated to each other positively. both the exchangeable fraction and the carbonate fraction of either Cu or Zn or Pb exist only in relatively small amounts, while the reducible fractions are excellent scavengers for all measured heavy metals. The measured oxidizable fraction exhibits higher retention capability for Zn and Cu, but lower retention capability for Pb. Similar distribution patterns of Cu, Zn, Pb appeared between the sediments of *Aegiceras corniculatum* community habitats and the sediments of *Limonium sinense* community habitats, and the same similarity appeared in the sediment between *Alternanthera philoxeroides* communities and *Phragmites communis* communities. Most of the heavy metals may be bound to the immobile fraction and unavailable to plants. *Alternanthera philoxeroides* has the best ability to accumulate Cu, Zn and Pb compared with *Aegiceras corniculatum, Limonium sinense* and *Phragmites communis* under natural conditions. *Phragmites communis* was quite tolerant to Zn and Pb and had a good capability to transfer Zn and Pb. *Aegiceras corniculatum* is a useful species in resisting heavy metal pollution, rather than an indicator of contamination. More action should be taken to deal with Zn and Pb pollutants in Quanzhou Bay's wetlands. Among the nourishment elements, K content in the soil of coastal Quanzhou Bay is higher. The bioaccumulation abilities of *Alternanthera philoxeroides, Alternanthera sessilis, Solanum nigrum, Phragmites australis* are stronger than other researched plants, and these plants present potential capacity of phytoremediation for the heavy-metal contaminated soil. The ability of absorbing and transferring K in *Chenopodium album* is high, and K contents of their roots, stems or leaves are highest in all research plants included in the study. The nitrogen contents of leaves in *Basella rubra, Alternanthera sessilis,*

Phragmites australis, Chenopodium album are higher, but the phosphorus contents of *Basella rubra, Alternanthera sessilis* are much lower, and the ratios of N/P are extremely high. There is no significant correlation between the contents of N, P, K with the contents of Cu, Zn, Pb in the coastal plants grown up under the natural condition of Quanzhou Bay.

2 The carbohydrate metabolism including the activities of α-mylase and the content of chlorophyll in *Aegiceras corniculatum* seedlings were affected by both their exposure time and increasing Pb concentratin in the sediment soils. The activities ofα-mylase in the seedlings with 15 d Pb stress are higher than that of 3 d Pb stress, With the lower concentrations under 2 000 mg/kg Pb in the sediments, the activities of α-mylase in the seedlings were higher than the control group without Pb stress, while the activities decreased significantly with Pb concentration up to 3 000 mg/kg in the sediment. It showed the damage to synthesizing chlorophyll with the treatment of Pb concentration up to 3 000 mg/kg, and the damage to chlorophyll a is much more pronounced than that to the chlorophyll b.

3 There were some effects on nitrogen metabolism in the roots, the stems, and the leaves of *Aegiceras corniculatum* seedlings cultivated for 30 days in nutrient solution containing Pb^{2+}. The NADH-GOGAT activities in *Aegiceras corniculatum* seedling roots，stems and leaves decreased under Pb^{2+} stress in nutrient solution，while the content of soluble proteins and MDA increased. The GS activity of the roots and stems decreased due to Pb^{2+} treatments of the concentrations with the range of 0.1 to 2 $mmol \cdot L^{-1}$，while the GS activity of the leaves increased and higher than that of the control experiment group, but which were inhibited by Pb^{2+} with a higher concentration of $2mmol \cdot L^{-1}$. Whether Pb^{2+} was added or not to the nutrient solution, the GS activitiy of the leaves was higher than those of the roots and stems, while the GOGAT activitiy of the roots was higher than those of the leaves and stems. GS and NADH-GOGAT in roots was more sensitive than those in stems and leaves to Pb^{2+} stress, thus Pb^{2+} had the most effect on primary nitrogen metabolism of root. It was also suggested that there is some negative correlation between the content of MDA and the NADH-GOGAT activity or the GS activity.

4 The SOD activity of the leaf is strongest of all vegetative organs of *Aegiceras corniculatum* seedlings, and the activity of the root is weaker than that of the stem

and the leaf. There are Cu·Zn–SOD isozymes in *Aegiceras corniculatum* and there is no organ specificity of SOD isozymes existing in *Aegiceras corniculatum* habitated in the natural wetland condition of Quanzhou Bay. This presents a similar strong mode between SOD isozymes and SOD activity of *Aegiceras corniculatum* vegetative organ, that is SOD isozymes and SOD activity in the leaf and stem are stronger than those of the root.

5 Extracting RNA with CTAB is a good method to extract RNA of mangrove species *Aegiceras corniculatum*. A part of sequences of Cu,Zn-SOD cDNA with 278bp was cloned from the young stem tissue of *Aegiceras corniculatum* (Genebank accession number DQ913822). The cDNA sequence is a portion of the open reading frame（ORF）near the C-Terminal Domain. An amino acid sequence comparison of *Gossypium hirsutum* and other plant species proteins shows that the Cu,Zn-SOD sequence is highly homologized with those cloned from other plant species, the identity can achieve 93%.

6 Increasing SOD activity of *Aegiceras corniculatum* stem tips induced by a certain range of Cd^{2+} and Pb^{2+}concentration might show a adaptive protection. Cu, Zn-SOD gene showed a good ability to resist Pb^{2+} stress but sensitivity to Cd^{2+} stress. The expression level of Cu, Zn-SOD gene in *Aegiceras corniculatum* stem tips increased notably with 40μmol•L^{-1} Cd^{2+} stress. This existed an antagonistic action between Pb^{2+} and Cd^{2+} in the SOD metabolization of *Aegiceras corniculatum* stem tips.

7 A part of sequences of PM H^+-ATPase cDNA with 323bp was cloned from the young stem tissue of *Aegiceras corniculatum.* The PM H^+-ATPase activities of *Aegiceras corniculatum* vegetative organs from high to low order as follows: stem > root > leaf. The expression level of PM H^+-ATPase gene in *Aegiceras corniculatum* leaf significantly higher than that of root and that of stem, while there was no obvious difference between the expression level of root and that of stem. The expression levels of PM H^+-ATPase gene in *Aegiceras corniculatum* leaf increased by Cd^{2+}stress or Pb^{2+} stress or the combined Pb^{2+} and Cd^{2+} stress, but all the expression levels in the root decreased under those stresses. The expression level of PM H^+-ATPase rapidly increased under Cd^{2+} stress with higher concentration, while the sensitivity to Cd^{2+} stress would become weak as Pb^{2+} entering the stress system.

8 The chlorophyll contents in *Eichhornia crassipes* with treatments of 0.1mmol·L^{-1} ~

2mmol·L^{-1} Pb^{2+} are higher than the comparison group with no Pb^{2+} stress significantly, but the ratios of Chlorophyll a/ Chlorophyll b decrease remarkably. The Hill reaction activities of *Eichhornia crassipes* leaves with Pb^{2+} treatment of lower concentration are higher than that of the comparison group considerably，but the activities are inhibited with Pb^{2+} at higher concentration(≥0.5mmol·L^{-1} Pb^{2+}), and the inhibition of their activities were more and strongly with increasing concentration of Pb^{2+}. The results of observing Chlorophyll fluorescence microstructure of *Eichhornia crassipes* showed that higher concentration Pb^{2+} stress on *Eichhornia crassipes* can damage the chloroplasts structure partly, including destroying the thylakoid membrane, and relaxing the dense of granum. MDA contents of *Eichhornia crassipes* leaves decrease when the plants were under low concentration Pb^{2+} stress, which would result from the new balance between producing and removing active oxygen built by the antioxidant protection system of *Eichhornia crassipes* leaves, but more serious peroxidation of membrane-lipid occurred when treated with higher concentration of Pb^{2+}.

9 The account of the Micro-organisms of *Eichhornia crassipes* root and the value of DO,COD were influenced by Pb^{2+} stress depended on not only time ,but also the concentrations. The amounts of every micro-organisms in seedling growing in the treated water for 27 days are fewer than those of 3 days caused by Pb^{2+} pollution with a series concentration from 0.1mmol/L to 2.0 mmol/L, and they all showed a decrease tendency after the first increase. Bacterial colony was dominant，whose tolerant concentration of Pb^{2+} was 0.25mmol/L and those of *Fungi was* 0.50mmol/l. There was trends that DO increased while COD decreased of each water sample with the increased stress time. However, at the same time, as the concentration of Pb^{2+} increased, the DO value showed a decrease after the first increase, while the COD value was in reverse with DO, and there was a relationship between DO ,COD and the growth of micro-organisms in the water.

10 96h LC_{50} of Cd^{2+} on *Phascolosoma esculenta* is 35.84 μg·g^{-1} (fresh mud). The SOD and CAT activities of enteron were higher than those of humor, while the concentration of MDA was lower than that of humor. In the exposure of 0.4 μg·g^{-1}～80 μg·g^{-1} Cd^{2+}, there were different responses of antioxidant enzymes activities and MDA content between *Phascolosoma esculenta* humor and

enteron, and humor was more vulnerable than enteron with Cd^{2+} exposure.

11 The BaP content of *Phascolosoma esculenta* body wall was obviously increased under BaP stress with 100～10000 ng / g dry sediment. *Phascolosoma esculenta* showed a strong ability to resist BaP stress but a low ability to accumulate BaP. The part cDNA sequences of Mn-SOD (Genebank accession number EF062359, protein_id=ABN13920.1) and 18srRNA were cloned and identified. Amino acid sequence comparison of *Phascolosoma esculenta* and aquatic species proteins shows that the Mn-SOD sequence is highly homologized with those cloned from other species, the identity can achieve 68%. The sequence identity of the 18srRNA cDNA between *Phascolosoma esculenta* and some other *Phascolosoma* species can achieve 100%.

12 The exposure of 100 ～10000 ng BaP·g^{-1}dry sediment brought different effects on both the total SOD activities and the Mn-SOD activities in *Phascolosoma esculenta* body wall. Change in SOD activities did not show a certain rule as the change in BaP concentration, but SOD activities showed the similar tendency with the BaP exposure time. The SOD activities reached the peak value when the exposure time reached 4 d, then the activities gradually decreased with increasing exposure time. The proportion of Mn-SOD in the total SOD increased with the exposure time. Mn-SOD gene was sensitive to BaP, and the expression level rapidly increased and reached the peak value in 4 h, then the amplitude modulation decreased, while the expression level of Mn-SOD gene showed decrease tendency when the exposure time reached 2 d. The response of SOD gene level to BaP was sensitive than those responses to SOD physiological activity. If thinking over applying some biomarker for the pollution, the molecular response of SOD gene may be easier to alarm the earlier pollution than the physiological response of SOD activity.

Keywords: the wetland of Quanzhou Bay, *Aegiceras corniculatum*, *Eichhornia crassipes*, *Phascolosoma esculenta*, toxicological response, chemical pollution, Cu,Zn-SOD, Mn-SOD, PM H^+-ATPase

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附图6　福建泉州湾河口湿地省级自然保护区调整后主要动物分布图