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外源镉在秋茄-土壤系统中的稳定及植物效应

Stabilization and plant responses of *Kandelia obovata*-soil system to the exogenous cadmium

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中文摘要

红树林湿地土壤（沉积物）重金属污染日趋严重，为了探讨利用红树植物稳定重金属镉的可行性以及红树植物对外源镉的响应机理，选择对重金属耐性较强的秋茄(*Kandelia obovata*)为研究对象，利用根际箱在自然透光的温室中进行培养，对比研究了不同浓度（0、12.5、25、50、100 mg kg⁻¹）的外源可溶性镉在秋茄-土壤系统中的迁移转化、稳定及植物效应。对镉胁迫下秋茄的生理生态特征以及根阳离子交换量、铁锰氧化膜对镉的稳定，镉在秋茄植株中的形态分布、土壤镉的形态转化、土壤的 DTPA（二乙烯三胺五乙酸）提取态镉和总镉等方面进行了系统的研究。研究的主要结论有：

1. 镉胁迫秋茄体内镉的结合形态和区域化呈现明显差异。(1) 通过分步提取发现幼叶镉的结合形态多样化，其中主要以乙醇提取态 (F_{Ethanol})、醋酸提取态 (F_{HAC})、盐酸提取态 (F_{HCl}) 为主；成熟叶镉 F_{Ethanol} 和 F_{HCl} 两者百分含量之和超过95%，其中 F_{HCl} 的含量百分比超过50%。幼叶生长过程中，叶中镉 F_{Ethanol} 和 F_{HCl} 明显增加，其他结合态减少。(2) 茎和胚轴的镉含量随着镉处理浓度增加先增加后减少，以 F_{Ethanol} 为主。(3) 贮气根的镉含量随着处理浓度没有显著性差异，主要以 F_{Ethanol}、F_{HCl} 为主；细根的镉含量随着镉处理浓度呈单峰曲线变化，在 50 mg kg⁻¹ 时达到最大值，主要是以 F_{Ethanol}、F_{HAC}、F_{HCl} 为主。(4) 贮气根铁锰氧化膜中镉含量随着镉处理浓度也呈单峰曲线变化，细根铁锰氧化膜中镉含量与镉处理浓度显著正相关。(5) 秋茄各部位镉的富集系数随着镉处理浓度的增加而减少。细根的镉含量最高，镉富集系数最大，其次是成熟叶，根系对镉有较强的富集作用，成熟叶的凋落减少镉的胁迫。(6) 秋茄较强的耐镉能力与镉的亚细胞分布密切相关。功能相对重要的部位如细胞器镉的含量相对较少，从而表现出对镉的选择性分配。细胞壁的镉含量远大于细胞器和细胞液的含量，是主要的镉积累部位。秋茄体内的草酸根、磷酸根、单宁等与镉结合，根系铁锰氧化膜吸附镉以及细胞壁累积了大部分的镉从而对重金属镉具有较强的耐性。

2. 秋茄在镉胁迫下产生不同的植物效应，渗透调节物质变化明显。(1) 镉处理秋茄茎、叶、胚轴和根的生物量随着镉处理浓度增加而减少，相对于对照组来说

镉处理组的茎、叶、胚轴的生物量的减少都大于根，反映了秋茄主要通过减少地上部分的生物量对镉胁迫的响应。(2) 镉胁迫秋茄渗透调节物质变化明显。秋茄叶可溶性蛋白和脯氨酸的含量随着镉处理浓度呈单峰曲线变化，叶可溶性糖、丙二醛含量随着镉处理浓度增加而增加；对照组秋茄叶的有机酸含量明显的高于各个镉处理组，茎的有机酸各处理之间没有显著性差异，胚轴的有机酸镉处理组之间没有显著性差异，但显著大于对照组，贮气根和细根的有机酸随着镉处理浓度的增加而增大，与镉胁迫浓度呈显著正相关；镉处理使秋茄各个部位的草酸根离子增加，产生的草酸一方面与秋茄植物体内的镉相结合，降低镉的活性，另一方面秋茄根系分泌的草酸钝化可溶性的镉，使可溶性镉得到稳定。秋茄通过生物量和可溶性蛋白、可溶性糖、脯氨酸、丙二醛、有机酸等渗透调节物质的变化来对镉胁迫的响应。

3.在镉胁迫下，秋茄的元素组成发生了变化，与镉之间的相互作用明显。(1) 细根和贮气根阳离子交换量随着镉处理浓度发生一系列的变化说明镉影响了秋茄对营养物质的吸收能力。(2) 随着镉处理浓度的增加，秋茄植物体的镉与铁、钾、镁、铝、铅和砷呈显著正相关 ($P < 0.01$)，表现为协同作用。铁、钾、镁、铝、铅和砷也主要集中在根部。同时这七种元素之间相互之间也具有显著正相关 ($P < 0.01$)，共同影响秋茄的生长。(3) 叶、茎、胚轴的全磷随着镉处理浓度增加先增加后减少，达到最大值分别在镉处理浓度为50、25、12.5 mg kg^{-1} ；细根的全磷随着镉处理浓度增加而减少，镉胁迫组的全磷含量比对照组小。(4) 秋茄叶和根全硫含量随着镉处理浓度增加迅速增加；镉处理组茎全硫远大于对照组，但随着镉处理浓度增加反而下降；胚轴全硫含量随着镉处理浓度增加先增加后减少。镉处理组的秋茄全硫大于对照组，镉处理促进秋茄对土壤中硫的吸收，体现了硫在镉胁迫中具有一定的作用。

4.秋茄-土壤系统将外源可溶性镉稳定下来，根际土壤稳定量大于非根际土壤。(1)根际土壤镉 DTPA 提取率小于同一处理下的近根际、远根际和非根际，根际区域秋茄能够将可溶态的镉较多地转化为植物不可以利用的镉，浓度越高根际区域稳定的镉相对越多。(2) 同一处理组中，根际区域土壤 pH 值比其他区域低。其中根际区域的土壤 pH 值比非根际区域低 0.3~0.5。镉胁迫处理组的土壤 pH 值比对照组低，镉浓度越高 pH 值越低，镉胁迫下秋茄根系分泌较多有机酸，

尤其是草酸将可溶性的镉转化为不可溶的草酸镉， H^+ 增加，pH 值越低，生物有效性减少。(3)根际土壤可溶性的镉含量由原来的 12.5、25、50、100 $mg\ kg^{-1}$ 变为 0.88、5.14、10.49、35.51 $mg\ kg^{-1}$ 。随着镉处理浓度的增加，稳定下来的镉依次增加。根际的可溶态的镉含量小于近根际、远根际和非根际，根际的稳定形式与非根际的不同，根际环境更有利于可溶性镉稳定下来。秋茄根系分泌草酸等有机酸及其它根系分泌物结合根际微生物钝化可溶性镉，降低镉的生物有效性，改变镉的形态等减少进入秋茄体内镉的含量，从而秋茄能够在高镉污染土壤中生长。因此，对于红树林湿地的镉污染土壤可以利用植物稳定的方法防止重金属镉迁移扩散导致更大范围的污染。

关键词：秋茄 植物-土壤系统 镉形态 植物稳定

Abstract

To reveal the response mechanism of mangrove-soil system to the heavy metal pollution, the migration and transformation of cadmium (Cd) had been studied in a plant-soil system planted with *Kandelia obovata* in the artificial rhizoboxes. In this research, the subcellular distribution and speciation distribution of Cd in different *Kandelia obovata* organs were determined under different Cd concentration treatments (0、12.5、25、50、100 ppm) ; the physiological responses of *Kandelia obovata* to the different concentration of Cd stress were discussed; and the relationships among diethylenetriaminepentaacetic acid (DTPA) extractable Cd content, iron plaque content on plant root surface, cation exchange capacity (CEC) of the roots, etc with Cd accumulation by plant were systemically explored. The major conclusions were summarized as following:

1. Significant differences were found for Cd distribution and existing forms in plants under the study treatments. (1) Sequential extraction of Cd in various parts of plants showed that in the young leaves, the greatest amount of Cd was found in extraction solution of 80% ethanol, 2% acetic acid (HAC) and 0.6 M hydrochloric acid (HCl). While in the mature leaves, the highest value of Cd accumulation was recorded in the fractions extracted by 80% ethanol and 0.6 M HCl. With the growth of leaves, the amount of Cd in the extraction solution of 80% ethanol and 0.6 M HCl significantly increased accompanied by the decreasing of the other forms. (2) With the Cd concentration increasing from 0 to 50 ppm in the study treatments, Cd contents in the stems and hypocotyls increased gradually; however under 100 ppm Cd treatment, Cd contents in the stems and hypocotyls were lower than that of 50ppm. Most of accumulated Cd in both the stems and the hypocotyls existed in the fraction extracted by 80% ethanol. (3) No significant difference was found in Cd concentrations of the aerating roots under different treatments, and Cd accumulated mainly existed in extraction solution of 80% ethanol and 0.6 M HCl. Cd concentration in the fine roots

increased with the Cd treatment concentration increasing from 0 to 50 ppm; the highest value of Cd accumulation was recorded in the fractions extracted by 80% ethanol, 2% HAC and 0.6 M HCl. (4) There was a positive correlation between Cd contents in the iron plaque of the fine roots and Cd concentration of treatments. While for the aerating roots, peak value of Cd content in the iron plaque appeared under 50 ppm Cd treatment. (5) The enrichment factors of different parts of *Kandelia obovata* decreased with the Cd concentration increasing. Fine roots had the highest Cd content and the biggest enrichment factor, followed by the mature leaves. (6) Cd concentration in the cell walls of plant leaves were significantly higher than that in the cell organelles and cytolymph, the cell wall was the main distribution site, followed by the cytolymph, and organelles combined the lowest Cd. Most of the accumulated Cd was distributed in the cell wall which might increase the endurance ability of *Kandelia obovata* to Cd stress. In conclusion, Cd accumulated by *Kandelia obovata* may be absorbed in iron plaque on the root surface, deposited in the cell wall, and combine with secondary metabolites (oxalate, phosphate etc.) in plant tissue, which made *Kandelia obovata* endurance high concentration Cd stress.

2. Cd stress had great influence on both biomass and physiological activities of *Kandelia obovata*. (1) Under study condition, the biomass of *Kandelia obovata* decreased significantly with the Cd concentration increasing. Meanwhile, biomass of the aboveground parts (stems, leaves, hypocotyls) reduced more compared to biomass of roots, which indicated that aboveground biomass of *Kandelia obovata* was more sensitive to the Cd stress. (2) The content of malondialdehyde (MDA) and osmotic adjustment in leaves of *Kandelia obovata* changed under Cd stress. Soluble protein and proline contents in the leaves all increased with Cd concentration increasing from 0-50 ppm; and when stressed with 100 ppm Cd, their contents reduced significantly. However, the contents of soluble sugar and MDA in the leaves increased gradually with the increasing of Cd concentration under study. (3) Compared to the control treatment (CK), organic acids contents in the leaves decreased significantly under Cd stress; while organic acids contents in the hypocotyls increased significantly. Cd stress also induced a significant increase of oxalic acid content in the leaves compared to the

CK. (4) Oxalic acid of the parts of *Kandelia obovata* was higher than that of the CK under Cd treatment. On one side, oxalic acid reduced the bioavailability of cadmium through the combination of cadmium in plant tissues, on the other side *Kandelia obovata* stabilized the soluble cadmium by the oxalic acid in the root exudates. *Kandelia obovata* responded to cadmium stress through the change of the biomass and soluble protein, soluble sugar, proline, MDA, organic acids, etc..

3. Element concentration in *Kandelia obovata* tissues changed under Cd stress and influenced each other with Cd. (1) Cation exchange capacity of fine roots and aerating roots changed with Cd treatment and this could reflect the impact of the Cd on absorption capacity of nutrients in plant tissues. (2) The Fe, K, Mg, Al, Pb and As concentrations significantly ($P < 0.01$) increased with increasing Cd supply. It illustrated the synergetic effect of elements in plant tissues. (3) The maximum phosphorus contents of the leaves, stems and hypocotyls were found with the Cd treatments of 50, 25, 12.5 ppm respectively. Total phosphorus content of the fine roots decreased with the increasing Cd concentrations and the total phosphorus contents under Cd treatment were smaller than that of the control. (3) Total sulfur contents of the leaves and roots increased rapidly with the Cd concentration increasing. Total sulfur contents of the stems were higher than that of the control. In hypocotyls, the total sulfur decreased with the increasing Cd concentration. The total sulfur contents under Cd treatments were higher than that of control.

4. The soils with different concentration of Cd changed after the growth of *Kandelia obovata*. Stabilization of Cd in the rhizosphere was higher than that of the bulk soil. (1) Under Cd treatments, the DTPA extractable rate of Cd in the rhizosphere was less than the other three regions (near rhizosphere, far rhizosphere and bulk soil). The maximum difference between the total Cd and the DTPA extractable Cd was found under 100 ppm Cd treatment. This indicated *Kandelia obovata* could turn the soluble Cd into unavailable Cd. (2) Under the same Cd treatment, pH value of rhizosphere was lower than that of the other regions. The soil pH value of rhizosphere was lower than the non-rhizosphere region by 0.3-0.5. The soil pH under Cd treatment was lower than that of the control, pH values reduced with increasing Cd concentration. (3)

Soluble Cd in mangrove wetland soils changed greatly after 150 days of planting, the most change was in the rhizosphere soil, from the initial 12.5, 25, 50, 100 ppm to 0.88, 5.14, 10.49, 35.51 ppm, regardless of the background value of soil, leaving only 7.04, 20.56, 20.98, 35.51% of the original, respectively. The rhizosphere played a well stabilization to the soluble Cd. With the Cd concentration increased, the stabilized Cd was increased and the proportion of stabilized Cd reduced. Soluble Cd increased from the rhizosphere to non-rhizosphere, the stabilization of Cd in rhizosphere was better than non-rhizosphere. The stable form of rhizosphere and non-rhizosphere were different, and the rhizosphere environment was more conducive to the stability of Cd. *Kandelia obovata* could reduce the bioavailability of cadmium through secretion of oxalic acid and other root exudates, which could immobilize the soluble cadmium by the interaction with the rhizosphere microorganisms and reduce the content of Cd in the plant. *Kandelia obovata* could survive in the soil of Cd pollution. Therefore, we could cultivate plants to deal with the mangrove wetlands of Cd pollution by the way of phytostabiliation, and it would provide a feasible way to prevent the migration and spread of Cd.

Keywords: *Kandelia obovata*; plant-soil system; cadmium speciation; phytostabiliation

第一章 前言

红树林是生长在热带、亚热带海岸潮间带的木本植物群落，是河口海湾生态系统重要的初级生产者，具有防汛、防浪、防风暴、保护堤岸、绿化及净化大气和水体等功能，对维护海岸生态平衡起着十分重要的作用^[1]。在2004年印度洋大海啸之后，人们认识到红树林是一个防御海啸无法替代的天然屏障^[2]，但红树林的作用远不只如此。随着江河流域工农业的发展与沿岸城市人口与经济的不断增长，人类对资源的需求量不断加大，各种含有重金属的废弃物不断地输入到环境中。大量排放的污染物汇集于河口、海湾区，使这些地区的重金属污染日趋严重，特别是直接向红树林区倾污排废的地区，重金属污染物大量汇集，严重影响了红树林生态系统的正常发展^[1]。红树林湿地生态系统中的重金属污染问题已引起国际社会和国内外生态学者们的极大关注，并对此进行了大量研究^[3]，有毒重金属离子的有效去除与如何利用植物稳定技术就成为一项富于挑战性的工作。植物稳定表现为技术措施简单，成本低，治理的原位性，对土壤扰动少，具有不可替代的优势而倍受关注，并逐步走向商业化^[4]。重金属镉容易生物富集，毒性大，进入食物链最终危害人类健康，也是红树林湿地比较典型的重金属污染物^[5]。

1.1 重金属镉的性质和危害

镉在自然界中的分布很广，在地壳中镉含量为 $0.15\sim 0.20\text{ mg kg}^{-1}$ ，在海水中为 $1.1\times 10^{-4}\text{ mg kg}^{-1}$ ，在河流湖泊中为 $1.0\times 10^{-4}\sim 10.0\times 10^{-4}\text{ mg kg}^{-1}$ ，在空气中为 $2.0\times 10^{-6}\sim 5.0\times 10^{-6}\text{ mg kg}^{-1}$ ，在土壤中大于 1 mg kg^{-1} 。在通常情况下，植物中镉含量比较低，不超过 1 mg kg^{-1} 。植物体中镉含量受许多因素的影响，如土壤pH值、植物种类及部位。镉在自然界中都以化合物的形式存在，主要矿物为硫镉矿(CdS)，与锌矿、铅锌矿、铜铅锌矿共生，浮选时大部分进入锌精矿，在焙烧过程中富集在烟尘中。在湿法炼锌时，镉存在于铜镉渣中。镉是银白色有光泽的金属，熔点 320.9°C ，沸点 765°C ，相对密度8.642，有韧性和延展性。镉在潮湿空气中缓慢氧化并失去金属光泽，加热时表面形成棕色的氧化物层。高温下镉与卤素反应激烈，形成卤化镉，也可与硫直接化合，生成硫化镉。镉可溶于酸，但不溶于碱，镉的氧化物价态为+1、+2，氧化镉和氢氧化镉的溶解度都很小。可

溶态镉以离子态 Cd^{2+} 或络合物形式 CdCl_4^{2-} 、 $\text{Cd}(\text{NH}_3)_4^{2+}$ 、 $\text{Cd}(\text{HS})_4^{2-}$ 等存在于土壤中，容易进入植物体引起植物的形态及生理发生变化^[7]。

镉是生物毒性最强的重金属元素之一，在环境中的化学活性强、移动性大、毒性持久^[8]。同时镉污染具有隐蔽性、长期性和不可逆性^[9,10,11]。镉为植物生长的非必需元素，植物对镉的过量吸收将对植物本身造成严重伤害。镉污染不仅使土壤肥力退化，作物产量和品质下降，水质变坏，而且通过食物链进入人体时就会直接影响和危及人类的健康^[12,13]。

1.2 红树林湿地系统的特点

红树林 (Mangrove) 是自然分布于热带、亚热带海湾河口潮间带，其土壤大多是由细质颗粒的沉积物组成的富含有机质、无结构的土壤，表现为高水分、高盐分、缺少氧气而含量大量还原性物质的特征^[1]，在高盐、频繁地周期性水淹环境中，红树植物进化出了一系列的适应特征，如聚盐、拒盐、泌盐、特化根、胎生等。红树林维护着该区域生物物种的多样性，是河口生态系统的初级生产者，是具有维护海岸生态平衡作用的特殊生态系，蕴藏着丰富的生物资源和生物多样性^[1]。

1.3 红树林湿地重金属污染研究

红树林生态系统是处于陆地和水生生态系统交错带之间的湿地生态系统，属于生态脆弱敏感带。红树林湿地具有过滤污染物的生态功能及潜在的污水净化能力，对来自陆地的污染物有缓冲作用。

1.3.1 重金属对红树植物的影响

非必需重金属和过量的必需重金属对植物都是有害的，可能会影响从种子萌发到生长发育等多个生理过程，进而导致植物生长受到抑制、发育异常甚至中毒死亡。红树林土壤中过量的重金属元素对红树植物的生长是一种胁迫或者说是逆境条件，这种胁迫对红树植物的影响因重金属种类、植物种类以及器官组织的不同而异^[14,15]，关于红树植物对重金属胁迫的生长响应的研究开展得较早。大量研究表明，红树植物对重金属污染能忍受一定程度的重金属胁迫，但高浓度的重金

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