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可生物降解螯合剂 GLDA 诱导东南景天修复重金属污染土壤的研究

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摘要:化学强化的植物提取技术可以有效地去除污染土壤的重金属。用盆栽试验的方法,研究了可生物降解螯合剂谷氨酸N,N-二乙酸(GLDA)不同用量或者与乙二胺四乙酸(EDTA)、柠檬酸复配使用对超富集植物东南景天吸收土壤重金属 Cd、Zn和Pb的影响。结果表明,施用GLDA提高了东南景天对土壤 Cd和Zn的提取效率,GLDA用量为2.5 mmol·kg⁻¹时东南景天对Cd和Zn的提取量最高,分别是对照的2.5 倍和2.6 倍。螯合剂的总用量为5 mmol·kg⁻¹时,EDTA、柠檬酸分别与GLDA复配,未能进一步促进东南景天对土壤 Cd和Zn的提取。上述结果表明,可生物降解螯合剂GLDA在诱导植物修复重金属污染土壤特别是Cd和Zn污染土壤具有明显潜力。

关键词:植物提取; GLDA; 东南景天; 重金属; 可生物降解

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Enhanced Phytoextraction of Heavy Metals from Contaminated Soils Using Sedum alfredii Hance with Biodegradable Chelate GLDA

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Abstract: Chemically enhanced phytoextraction by hyperaccumulator has been proposed as an effective approach to remove heavy metals from contaminated soil. Pot experiment was conducted to investigate the effect of application of the biodegradable chelate GLDA (L glutamic acid N,N-diacetic acid) at different doses or the combination of GLDA with EDTA (ethylenediamine tetraacetic acid) or CIT (citric acid) on the uptake of Cd, Zn and Pb by Sedum alfredii Hance (a Zn and Cd hyperaccumulator). Experimental results showed that GLDA addition to soil significantly increased the concentrations of Cd and Zn in Sedum alfredii Hance and its Cd and Zn phytoextraction compared to the control. Additionally, GLDA at 2.5 mmol·kg⁻¹ resulted in the highest phytoextraction, being 2.5 and 2.6 folds of the control for Cd and Zn, respectively. However, the combined application of GLDA + EDTA (1:1) and GLDA + CIT(1:1) and 1:3) at a total dose of 5 mmol·kg⁻¹ did not increase the phytoextraction of Zn and Cd, compared to the GLDA only treatment. Therefore, the biodegradable chelate GLDA could be regarded as a good chelate candidate for the phytoextraction of heavy metals of heavy metals from contaminated soils, particularly for Cd and Zn contaminated soils.

Key words: phytoextraction; GLDA; Sedum alfredii; heavy metal; biodegradable

植物提取是重金属污染土壤修复技术中最具前途的方式之一. 螯合剂可增加土壤重金属的可溶性,促进植物对重金属的吸收和积累,从而进一步提高植物对重金属的提取效率. EDTA(乙二胺四乙酸)具有较强的络合能力,是目前研究较多的螯合剂,但 EDTA 在环境中不易被生物降解,施入土壤中残留期较长,存在潜在的生态风险. 目前,包括土壤修复在内的许多行业均在研究寻找可生物降解的螯合剂 EDDS(乙二胺二琥珀酸)成为 EDTA 的主要替代品^[2,3],但是EDDS价格贵难以推广应用. 因此,寻找易生物降解的和环保安全的促进植物提取重金属的螯合剂或材料等成为必然.

近年来,一种可生物降解的环境友好螯合剂

GLDA(谷氨酸 N,N-二乙酸, L-glutamic acid N,N-diacetic acid)引起人们注意^[4]. GLDA 主要由植物原料制成,容易生物降解,在螯合能力方面,GLDA与EDTA的效率相当^[5,6]. GLDA用于淋洗污染土壤的重金属已有研究报道^[7,8]. 初步研究发现可生物降解螯合剂 GLDA 在诱导超富集植物东南景天提取土壤重金属方面具有潜力^[9]. 此外,对于螯合诱导植物修复的研究大多集中在某一种螯合剂对重金属土壤污染的治理上,螯合剂的联合使用的报道相

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对较少^[3,10]. 可生物降解的 GLDA 溶于水后经测定呈碱性,降低 pH 为 4、7 时可以提高 GLDA 对土壤淋洗去除率^[7],EDTA 和柠檬酸的溶液呈现酸性,本研究拟将 EDTA 和柠檬酸与 GLDA 复配后以期降低GLDA 溶液的 pH,研究复配后的 GLDA 能否促进超富集植物提取重金属. 鉴于上述,通过研究不同用量 GLDA 以及与 EDTA、柠檬酸复配对东南景天吸收和提取重金属的影响,以期为植物修复效率的提高提供强化措施和科学依据.

1 材料与方法

1.1 供试材料

供试土壤:采自广东省乐昌市重金属污染水稻 田的表层土,基本理化性质见表 1. 供试植物: 东南景天,取自浙江衢州古老铅锌矿山,Zn/Cd 超富集植物^[11-13],剪取大小均匀枝条直接扦插.

供试试剂:可生物降解螯合剂 GLDA(谷氨酸,N,N-乙酰乙酸四钠,GLDA-Na₄,47% 水溶液,产品名-Dissolvine® GL-47,购自阿克苏诺贝尔)、EDTA(乙二胺四乙酸二钠)(分析纯)、柠檬酸(分析纯).

1.2 试验设计

采用盆栽试验,土壤风干后过 5 mm 筛,分别采用尿素和 KH_2PO_4 (均为分析纯),用作基肥与土壤混匀,其用量分别为: N,100 $mg \cdot kg^{-1}$; P,80 $mg \cdot kg^{-1}$; K,100 $mg \cdot kg^{-1}$. 每盆装土 5 kg,2013 年12月11日种植东南景天,每盆 3 棵.

表 1 土壤基本理化性质

_	Table 1 Basic physico-chemical properties of the soils									
	项目	pН	有机质 /g·kg ⁻¹	CEC ¹⁾ /cmol·kg ⁻¹	总氮 /g·kg ⁻¹	总磷 /g·kg ⁻¹	总钾 /g•kg ⁻¹	Cd ∕mg•kg ⁻¹	Zn /mg·kg ⁻¹	Pb ∕mg•kg ⁻¹
	土壤	7. 59	37. 9	18.4	1.99	0. 73	16. 0	2. 173	1 070	1 024

1) CEC 表示阳离子交换量

试验设置 GLDA 不同用量 0(对照)、1.25、2.5、5、10 mmol·kg⁻¹ 土,以及 GLDA 与 EDTA、柠檬酸 (CIT)复配: 螯合剂总用量选用 5 mmol·kg⁻¹ 土,设置 GLDA: EDTA 为1:1(GLDA-EDTA, GLDA 和 EDTA 的用量均为 2.5 mmol·kg⁻¹)、GLDA: CIT 为1:1 (GLDA-CIT, GLDA 和 CIT 的用量均为 2.5

mmol·kg⁻¹)、GLDA: CIT 为1: 3 (GLDA1-CIT3, GLDA 的用量均为 1. 25 mmol·kg⁻¹; CIT 的用量均为 3. 75 mmol·kg⁻¹)等,共 8 个处理,每个处理 3 次重复.

2014年5月20日,相应的螯合剂溶于800 mL水,各螯合剂溶液的pH值见表2,小心浇灌到土壤表面,两周后收获东南景天.采集土壤样品.

表 2 不同用量的 GLDA 溶液的 pH 值

Table 2 The pH values of solutions with different GLDA doses

项目	GLDA-1. 25	GLDA2. 5	GLDA-5	GLDA-10	GLDA-EDTA	GLDA-CIT	GLDA1-CIT3
pH	9. 9	10	10. 3	10.6	7. 4	3.6	2. 3

1.3 样品的采集与分析

东南景天的采集和处理:用剪刀剪取地上部,称取地上部即为每盆东南景天产量.收获的东南景天用自来水冲洗干净,再用双蒸水漂洗3次,晾干,置于信封中烘干,粉碎贮存于封口袋中备测.

土壤和植物样品分析方法参照文献[14]进行, 土壤样品重金属形态分析采用 Tessier 连续提取 法^[15].

1.4 数据分析方法

所有数据用 Excel 软件处理,多重比较(Duncan)统计分析由 SAS 8.1 数据统计软件完成.

2 结果与分析

2.1 GLDA 处理对东南景天生物量的影响

单独使用 GLDA 时,随着其用量的增加,东南景天的生物量呈先增加后降低的趋势(图 1),其中 GLDA-2.5(2.5 mmol·kg⁻¹,以干土计,下同)处理的东南景天地上部生物量最高.高浓度的人工螯合剂对植物有毒害作用, GLDA 用量的过高(10 mmol·kg⁻¹)时,抑制了东南景天的生长. Luo 等^[3]通过盆栽试验表明,EDTA(5 mmol·kg⁻¹)处理显著降低玉米的生物量,EDTA施用量在12.5 mmol·kg⁻¹以上时,显著降低了烟草茎叶的干重^[16];

5 mmol·kg⁻¹ 的 EDDS 显著降低了东南景天的地上部干 重^[17]. 在 污 染 土 壤 上, 施 用 适 量 (2.5 mmol·kg⁻¹)的 GLDA 有助于东南景天的生长.

GLDA-EDTA 处理的东南景天生物量低于GLDA-5处理,低于GLDA-2.5处理。可见,GLDA与EDTA复配没有增加东南景天的生物量。GLDA-CIT处理的东南景天生物量低于GLDA-2.5处理,GLDA1-CIT3处理低于GLDA-1.25处理,GLDA与柠檬酸复配处理也没有增加东南景天的生物量。因此,需要进一步研究与GLDA复配的组合和比例等。

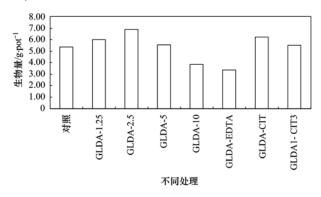


图 1 不同用量 GLDA 处理东南景天的生物量

Fig. 1 Biomass of Sedum alfredii Hance treated with different doses of GLDA

2.2 GLDA 处理对东南景天重金属含量的影响

在东南景天生长旺盛的时候,施加 GLDA 有助于植物最大程度地吸收被活化的重金属.施加 GLDA 提高了东南景天地上部 Cd 和 Zn 含量(表3),与对照处理相比,GLDA-2.5 处理的东南景天地上部 Cd 和 Zn 含量分别是对照的 1.9 倍和 2.0 倍.东南景天 Cd 和 Zn 含量并没有随着 GLDA 用量的增加而增加,主要是由于随着 GLDA 用量的增加东南景天的生长受到了影响,当每 kg 土使用 2.5 mmol GLDA 时东南景天地上部的 Cd 和 Zn 含量最高.

表 3 不同处理的东南景天重金属含量1)/mg·kg-1

Table 3 Heavy metal concentrations in Sedum alfredii

Hance after different treatments/mg·kg⁻¹

	Trance arrer amerent t	reatments/ mg kg	
处理	Cd	Zn	Pb
对照	31. 44 ± 3. 14b	1 947 ± 197e	10. 72 ± 0. 85b
GLDA-1. 25	45.93 ± 7.29 ab	$3.085 \pm 195 \mathrm{bc}$	11.77 ± 1.34 b
GLDA-2. 5	$58.55 \pm 4.07a$	$3972\pm452a$	14. 52 ± 1.68 b
GLDA-5	$46.66 \pm 6.11 ab$	$3~072~\pm202\mathrm{bc}$	20. 65 ± 4.76 b
GLDA-10	$51.68 \pm 5.96a$	$2810\pm41\mathrm{bcd}$	23. $34 \pm 4.38b$
GLDA-EDTA	$44.49 \pm 4.28 ab$	$3304 \pm 274 ab$	290. 3 ± 16 . $4a$
GLDA- CIT	32.45 ± 1.55 b	$2\;392\pm317\mathrm{cde}$	18. 67 \pm 2. 77b
GLDA1- CIT3	$32.77 \pm 6.30b$	$2162 \pm 178 \mathrm{de}$	$9.795 \pm 0.532b$

1)根据 Duncan 检验(P = 0.05),同列具有相同字母的数据间无显著性差异; CIT: 柠檬酸,下同

与GLDA-2.5 处理相比,GLDA-EDTA 和GLDA-CIT 处理没有提高东南景天 Cd 和 Zn 含量;与GLDA-1.25 处理相比,GLDA1-3CIT 处理没有提高东南景天天 Cd 和 Zn 含量. 因此,EDTA 和CIT 与GLDA 复配不能进一步提高东南景天 Cd 和 Zn 含量.

除 GLDA1-CIT3 处理外, GLDA 的施加提高了东南景天地上部 Pb 含量(表3). 只有 GLDA-EDTA处理显著高于其他处理, 其他处理间无显著差异, GLDA-EDTA 处理的东南景天 Pb 含量是对照处理的 27 倍. 主要是复配的 EDTA 的作用, 单独 EDTA处理的东南景天 Pb 含量和提取量高于 GLDA 处理^[9], 众多研究表明, 不同螯合剂中, 对 Pb 的螯合诱导效果最强的是 EDTA^[18-20]. 在强化植物提取土壤 Pb 方面, 目前还没有报道优于 EDTA 的螯合剂.

2.3 GLDA 处理对东南景天提取重金属的影响

东南景天对重金属的提取量用地上部的生物量 与重金属含量的乘积来表示,不同处理的东南景天 对重金属的提取量见表 4. GLDA-2.5 处理的东南 景天对 Cd 和 Zn 的提取量最高(表4),分别是对照 处理的 2.5 倍和 2.6 倍. 单独使用 GLDA 时,随着 其用量的增加,东南景天提取 Cd 和 Zn 的量呈先增 加后降低趋势,GLDA-2.5 处理的最高. 综合考虑东 南景天地上部的生物量、Cd 和 Zn 含量及提取量, 每 kg 土使用 2.5 mmol GLDA 的效果最佳. GLDA 在土壤中的生物降解率缺乏数据,在密封瓶试验 (OECD 301D)中,GLDA的28d生物降解率超过 60%;在固有生物降解(Inherent biodegradability)试 验中(OECD 302B), GLDA 的 21 d 生物降解率为 98%. 可见,GLDA 具有生物可降解性[5];同样可被 生物降解螯合剂 EDDS,在土壤中残留时间短、施用 EDDS 相对风险较小且可控^[21~23].

GLDA-CIT 和 GLDA1-CIT3 处理的东南景天提取 Cd 和 Zn 的量低于 GLDA-12.5、GLDA-2.5、GLDA-5 处理,说明柠檬酸的复配不能增加东南景天对 Cd 和 Zn 的提取. EDTA 与 GLDA 复配,影响了东南景天的生物量(图1),GLDA-EDTA 处理的东南景天地上部的 Cd 和 Zn 含量和提取量较低(表3和表4),EDTA 的复配未能提高 GLDA 促进东南景天提取 Cd 和 Zn. GLDA-EDTA 处理东南景天提取 Pb 量显著高于其他处理(表4),该处理东南景天地上部 Pb 含量是其他处理的 27 倍(表 3),主要是EDTA 螯合能力强的原因. 有研究报道多种螯合剂的适当复配可以起到协同或促进作用[10],本研究中GLDA 与 EDTA、柠檬酸等设置的复配比例数量较

表 4	不同用量 GI	DA 处理东南景天对重金属的提取量/mg·pot -1
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Table 4 Zn, Cd and Pb uptake by plants treated with different GLDA doses/mg·p

处理	Cd	Zn	Pb
对照	$0.1648 \pm 0.0178 cd$	10. 43 ± 1. 06e	0. 057 4 ± 0. 004 5b
GLDA-1. 25	$0.2762 \pm 0.0438b$	$18.55 \pm 1.17b$	$0.0708 \pm 0.0081b$
GLDA-2. 5	$0.4046 \pm 0.0281a$	$27.44 \pm 3.12a$	0. $100.4 \pm 0.011.6$ b
GLDA-5	$0.2582 \pm 0.0338 bc$	$17.00 \pm 1.12b$	0. $1143 \pm 0.0263b$
GLDA-10	0. 199 5 \pm 0. 023 0bcd	$10.85 \pm 0.16c$	$0.0901 \pm 0.0169b$
GLDA-EDTA	$0.1489 \pm 0.0143 d$	$11.06 \pm 0.92c$	$0.9715 \pm 0.0550a$
GLDA- CIT	$0.2026 \pm 0.0097 $ bcd	$14.94 \pm 1.98 \mathrm{bc}$	0. $116.6 \pm 0.017.3 \mathrm{b}$
GLDA1 - CIT3	$0.1809 \pm 0.0348 cd$	$11.94 \pm 0.98c$	$0.0541 \pm 0.0029b$

少,未能发现促进植物生长和吸收 Cd、Zn 的组合, 需要进一步研究.

2.4 GLDA 处理对土壤酸碱度及重金属含量和形态的影响

植物收获后,不同处理土壤 pH 见表 5,与对照相比施加 GLDA 后土壤 pH 值升高,其中 GLDA 用量最多的 GLDA-10 处理的 pH 最高,GLDA 的碱性也会一定程度上提高土壤 pH 值(表 2),但与其他

处理无显著差异,可能与土壤具有较强的缓冲能力, 螯合剂溶液的 pH 对土壤的 pH 影响不大有关,有研究发现螯合剂强化植物提取重金属并不取决于土壤 pH 的变化^[3].

不同处理土壤重金属全量见表 5. 不同处理重金属全量变化不大,与对照相比有的略有升高,这可能跟植物提取量占土壤起始重金属总量的比例小有关.

表 5 不同处理土壤 pH 和重金属全量/mg·kg-1

Table 5 Heavy metal contents and pH of soil after different treatments/mg·kg⁻¹

		1	8 8	
处理	рН	Cd	Zn	Pb
对照	$7.58 \pm 0.03 d$	$2.043 \pm 0.143 ab$	975. 0 ± 33. 4a	1 007 ±9b
GLDA-1. 25	$7.64 \pm .0.4 cd$	2.012 ± 0.094 ab	$1015 \pm 33a$	$1.016 \pm 17 ab$
GLDA-2. 5	$7.81 \pm 0.04 bc$	$2.035 \pm 0.048 ab$	$1.068 \pm 48a$	$1.033 \pm 3ab$
GLDA-5	7.87 ± 0.07 ab	1.930 ± 0.026 ab	$1015\pm21a$	$1.068 \pm 19a$
GLDA-10	$8.00 \pm 0.08a$	1. 988 \pm 0. 044 ab	997. 7 ± 25 . $2a$	$1.033 \pm 27 ab$
GLDA-EDTA	$7.75 \pm 0.04 bc$	2. $155 \pm 0.035a$	$1078 \pm 94a$	$1030 \pm 22ab$
GLDA- CIT	$7.81 \pm 0.03 \mathrm{bc}$	1. 881 \pm 0. 033b	$1.043 \pm 3a$	$1.044 \pm 8 \text{ab}$
GLDA1- CIT3	$7.74 \pm 0.06 $ bcd	1.873 ± 0.063 b	$1086 \pm 50a$	$1009\pm11{\rm b}$

图 2~4 显示不同处理土壤重金属形态分布,3 种重金属其交换态、碳酸盐结合态、铁锰氧化物结 合态、有机结合态和残渣态所占的比例不同,交换

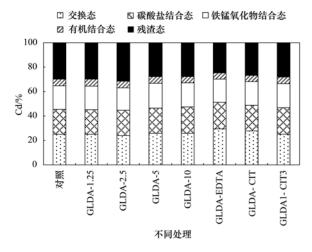


图 2 不同处理土壤 Cd 形态分布特征

Fig. 2 Speciation distribution of Cd in soil after different treatments

态 Cd 所占比例最大.

由图 2 可以看出, Cd 各形态在不同处理所占比

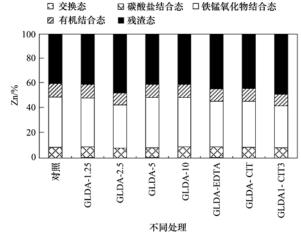


图 3 不同处理土壤 Zn 形态分布特征

Fig. 3 Speciation distribution characteristics of Zn in soil after different treatments

例相近. Cd 主要以交换态、碳酸盐结合态、铁锰氧化物结合态和残渣态为主,而有机结合态只占5%左右. GLDA-EDTA 处理的交换态、碳酸盐结合态、铁锰氧化物结合态 Cd 所占比例高于对照和其他处理. 对土壤 Zn,以铁锰氧化物结合态和残渣态为主,占80%以上(图3). 土壤 Pb 以铁锰氧化物结合态和残渣态为主(图4),占71%.3 种重金属均是GLDA-EDTA 处理的交换态比例高于其他处理,可能是残留 EDTA 的影响^[20].

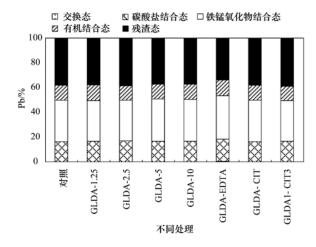


图 4 不同处理土壤 Pb 形态分布特征

Fig. 4 Speciation distribution characteristics of Pb in soil after different treatments

3 结论

- (1)施加 2.5 mmol·kg⁻¹的 GLDA 可以显著提高东南景天地上部的生物量和 Cd、Zn 浓度,进而提高了东南景天对土壤 Cd 和 Zn 的提取效率.
- (2) EDTA、柠檬酸与 GLDA 复配,未能进一步提高 GLDA 促进东南景天提取 Cd 和 Zn; EDTA 与 GLDA 复配使用,抑制了东南景天生长,但提高了东南景天地上部的 Pb 含量和总提取量.
- (3) 环境风险较小、可生物降解的螯合剂 GLDA 在强化植物修复重金属污染土壤特别是 Cd、Zn 污染土壤上具有明显的潜力.

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