

申 报	系列：教师系列教 学科研并重型
	专业：植物营养学
	职称：副教授

业绩成果材料

（申报人的业绩成果材料包括论文、科研项目、获奖以及其他成果等）

单 位（二级单位） 资源环境学院

姓 名 杨 旭

材料核对人：

单位盖章：

核对时间：

华南农业大学制

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奖状

杨旭老师：

在资源环境学院第九届教师教学基本功大赛中
荣获 二 等奖。

特发此状，以资鼓励。

华南农业大学资源环境学院

二〇二三年十二月



项目批准号	42107016
申请代码	D0701
归口管理部门	
依托单位代码	51027508A1549-2815



国家自然科学基金 资助项目计划书 (包干制项目)

资助类别：青年科学基金项目

亚类说明：

附注说明：

项目名称：丛枝菌根共生强化超富集植物龙葵提取土壤Cd的机制研究

资助经费：30万元 执行年限：2022.01-2024.12

负责人：杨旭

通讯地址：广东广州番禺大学城中山大学环境学院

邮政编码：510006 电 话：18811845469

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依托单位：中山大学

联系人：张迪 电 话：020-84115962

填表日期：2021年10月18日

国家自然科学基金委员会制



国家自然科学基金资助项目计划书填报说明 （包干制项目）

- 一、项目负责人收到《国家自然科学基金资助项目批准通知》（以下简称《批准通知》）后，请认真阅读本填报说明，参照国家自然科学基金相关项目管理办​​法和新修订的《国家自然科学基金资助项目资金管理办法》（以下简称《资金管理办法》，请查阅国家自然科学基金委员会官方网站首页“政策法规”栏目），按《批准通知》的要求认真填写和提交《国家自然科学基金资助项目计划书》（以下简称《计划书》）。
- 二、填写《计划书》时要科学严谨、实事求是、表述清晰、准确。《计划书》经国家自然科学基金委员会相关项目管理部门审核批准后，将作为项目研究计划执行、检查和验收的依据。
- 三、《计划书》各部分填写要求如下：
 - （一）简表：由系统自动生成。
 - （二）摘要及关键词：各类获资助项目都应当填写中、英文摘要及关键词。
 - （三）正文：
 1. 青年科学基金项目：如果《批准通知》所附“项目评审意见及修改意见表”中“修改意见”栏目没有修改要求的，只需选择“研究内容和研究目标按照申请书执行”即可；如果《批准通知》中上述栏目明确要求调整研究期限或研究内容等的，须选择“根据研究方案修改意见更改”并填报相关修改内容。
 2. 国家杰出青年科学基金和优秀青年科学基金：须选择“根据研究方案修改意见更改”，按下列提纲撰写：
 - （1）研究方向；
 - （2）结合国内外研究现状，说明研究工作的学术思想和科学意义（限两个页面）；
 - （3）研究内容、研究方案及预期目标（限两个页面）；
 - （4）年度研究计划；
- 四、资助经费相关要求：
 1. 资助经费批准时不再区分直接费用和间接费用。
 2. 项目负责人在提交计划书时需签署承诺书，承诺尊重科研规律，弘扬科学家精神，遵守科研伦理道德和作风学风诚信要求，认真开展科学研究工作；承诺项目经费全部用于与本项目研究工作相关的支出，不得用于与本项目研究无关的支出。
 3. 项目负责人提交计划书时，无需编制项目预算。项目资金由项目负责人自主决定使用，按照《资金管理办法》第九条规定的开支范围列支。有关管理费用的补助支出，由依托单位根据实际管理需要，在充分征求项目负责人意见基础上合理确定。绩效支出由项目负责人根据实际科研需要和相关薪酬标准自主确定，依托单位按照工资制度进行管理。其余用途经费无额度限制，由项目负责人根据实际需要自主决定使用。



4. 项目结题时，项目负责人根据实际使用情况编制项目经费决算，经依托单位财务、科研管理部门审核后，报自然科学基金委。依托单位应当在单位内部公开非涉密项目立项、主要研究人员、资金使用（重点是间接费用、外拨资金、结余资金使用等）、决算、大型仪器设备购置以及项目研究成果等情况，接受内部监督。
5. 自然科学基金委结合项目管理，对经费使用情况和依托单位管理情况定期开展抽查。



简表

项目负责人信息	姓 名	杨旭	性 别	女	出生年月	1990年08月	民 族	汉族
	学 位	博士			职称	博士后		
	是否在站博士后	是			电子邮件	yangx375@mail.sysu.edu.cn		
	电 话	18811845469			个人网页			
	工 作 单 位	中山大学						
	所 在 院 系 所	环境科学与工程学院						
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	电 话	020-84115962			网站地址	http://research.sysu.edu.cn/		
合作单位信息	单 位 名 称							
项目基本信息	项 目 名 称	丛枝菌根共生强化超富集植物龙葵提取土壤Cd的机制研究						
	资 助 类 别	青年科学基金项目				亚 类 说 明		
	附 注 说 明							
	申 请 代 码	D0701:环境土壤学				D0711:污染物环境行为与效应		
	基 地 类 别							
	执 行 年 限	2022.01-2024.12						
	资 助 经 费	30万元						



项目摘要

中文摘要:

重金属镉（Cd）是影响我国耕地土壤环境质量的首要无机污染物，严重影响土地安全利用和人体健康。我们前期研究表明丛枝菌根真菌（AMF）显著促进超富集植物龙葵对Cd的积累，但菌根如何增强龙葵重金属Cd提取的作用机制仍不清楚。本项目以菌根促进龙葵Cd积累的菌根际调控机制为研究主线，通过稳定同位素与转录组分析，明确AMF提高该共生体系对Cd获取的直接（吸收）与间接（促生）作用，并定量菌根吸收途径对Cd吸收的贡献。同时，通过多区室盆栽培养、X射线荧光光谱法以及高通量测序平台，系统深入分析龙葵-AMF共生体系根土界面关键环境因子变化对重金属Cd活化的作用，从根际与菌丝际理化参数、生物学特征及其对微生物“招募”互作的差异出发，揭示菌根强化超富集植物对重金属提取的菌根际调控过程。项目研究可为植物-微生物互作增强超富集植物修复能力，实现重金属污染的绿色修复提供理论依据。

Abstract:

Cadmium (Cd) is the primary inorganic pollutant that affects the cultivated land quality in China, and seriously affects the safe use of soil and human health. Our previous studies showed that arbuscular mycorrhizal fungi (AMF) significantly promoted Cd accumulation in the Cd-hyperaccumulator *Solanum nigrum* (*S. nigrum*), but the related mechanism is still unclear. This project focuses on the mycorrhizal regulation mechanism on promoting Cd accumulation in *S. nigrum*. Based on the previous work, the Cd stable isotope and the transcriptome technique will be used to prove the direct and indirect effects, and the contribution rate of the mycorrhizal absorption pathway to Cd accumulation in *S. nigrum*. And the effects of key environmental factors at the root-soil interface on the Cd activation in this symbiosis system will be identified by the multi-compartment pot culture system, the X-ray fluorescence spectrometry, and the high-throughput sequencing technique. The rhizosphere and mycelial mechanism regulating the Cd uptake of the *S. nigrum*-AMF system will be revealed, by analyzing the physical and chemical parameters, the biological characteristics of the rhizosphere and mycelium, and the difference in the microbial interactions. This project will provide a scientific basis for plant-microbe interaction to enhance the remediation ability of hyperaccumulators, and realize the green remediation of the heavy metal-contaminated soil.

关键词(用分号分开): 超富集植物; 镉; 丛枝菌根真菌; 菌丝; 植物-微生物互作

Keywords(用分号分开): Hyperaccumulator; Cadmium; Arbuscular mycorrhizal fungi; Hypha; Plant-microbe interactions



报告正文

研究内容和研究目标按照申请书执行。



国家自然科学基金项目负责人、依托单位承诺书

国家自然科学基金项目负责人承诺书

本人郑重承诺：我接受国家自然科学基金的资助，严格遵守中共中央办公厅、国务院办公厅《关于进一步加强科研诚信建设的若干意见》《关于进一步弘扬科学家精神加强作风和学风建设的意见》等规定，及国家自然科学基金委员会关于资助项目管理、项目资金管理等各项规章制度，在《计划书》填写及项目执行过程中：

（一）按照《批准通知》《国家自然科学基金资助项目计划书填报说明》的要求填写《计划书》，未自行降低、更改目标任务或约定要求，或缩减研究（研制）内容；

（二）树立“红线”意识，严格履行科研合同义务，按照《计划书》负责实施本项目（批准号：42107016），切实保证研究工作时间，按时报送有关材料，及时报告重大情况变动，不违规将科研任务转包、分包他人，不以项目实施周期外或不相关成果充抵交差；

（三）遵守科研诚信、科研伦理规范和学术道德，认真开展研究工作，对资助项目发表的论著和取得的科研成果按规定进行标注，反对无实质学术贡献者“挂名”，不在成果署名、知识产权归属等方面侵占他人合法权益，并如实报告本人及团队成员发生的违背科研诚信要求的任何行为；

（四）尊重科研规律，弘扬科学家精神，严谨求实，追求卓越，反对浮夸浮躁、投机取巧，不人为夸大学术或技术价值，不传播未经科学验证的现象和观点；

（五）将项目资金全部用于与本项目研究工作相关的支出，并结合科研活动需要，科学合理安排项目资金支出进度。

如违背上述承诺，本人愿接受国家自然科学基金委员会和相关部门做出的各项处理决定。

项目负责人（签字）：

年 月 日

国家自然科学基金项目依托单位承诺书

我单位同意承担上述国家自然科学基金项目，将保证项目负责人及其研究队伍的稳定和研究项目实施所需的条件，严格遵守国家自然科学基金委员会有关资助项目管理、项目资金管理和科研诚信管理各项规定，并督促实施。

依托单位（公章）

年 月 日



国家自然科学基金资助项目签批审核表

本 栏 目 由 自 然 科 学 基 金 委 填 写	<p>科学处审查意见：</p> <p>负责人（签章）： 年 月 日</p>
	<p>科学部审查意见：</p> <p>负责人（签章）： 年 月 日</p>

国家自然科学基金 资助项目准予结题通知

杨旭 同志：

您承担的国家自然科学基金项目：（丛枝菌根共生强化超富集植物龙葵提取土壤Cd的机制研究），批准号：（42107016）按有关规定已审核完毕，准予结题。

与本项目资助有关的后续成果，请您继续及时报送。

祝您在研究工作中取得更好的成绩！



课题编号：2025YFF1309301

密 级：公开

国家重点研发计划 课题任务书

课题名称：	喀斯特矿区适生植物的优选、生态配置与资源化利用
所属项目：	广西喀斯特矿区重金属污染的植物固定与生态修复技术与示范
所属专项：	典型脆弱生态系统保护与修复
项目牵头承担单位：	中国科学院地理科学与资源研究所
课题承担单位：	桂林理工大学
课题负责人：	王敦球
执行期限：	2025 年 10 月 至 2028 年 09 月

中华人民共和国科学技术部制

2025 年 11 月 01 日

0003YF 2025YFF1309301 2025-11-01 18:14:47



课题预算表

表B1 课题编号： 2025YFF1309301 课题名称： 喀斯特矿区适生植物的优选、生态配置与资源化利用 金额单位： 万元

序号	预算科目名称	金额
	(1)	(2)
1	一、中央财政专项资金	97.00
2	（一）直接费用	75.00
3	1. 设备费	
4	其中：购置设备费	
5	2. 业务费	60.40
6	3. 劳务费	14.60
7	（二）间接费用	22.00
8	二、其他来源资金	193.00
9	三、合计	290.00

注：1. 间接费用无需编制预算说明；2. 绩效支出在间接费用中无比例限制。承担单位在统筹安排间接费用时，要处理好合理分摊间接成本和对科研人员激励的关系，绩效支出安排与科研人员在课题工作中的实际贡献挂钩。



设备费——购置/试制设备预算明细表

表B2 课题编号: 2025YFF1309301

课题名称: 喀斯特矿区适生植物的优选、生态配置与资源化利用

金额单位：万元

填表说明：

- 1.设备分类：购置、试制；
- 2.购置设备类型：通用、专用；
- 3.试制设备不需填列本表（9）列、（10）列、（11）列、（12）列；
- 4.设备单价的单位为万元/台套，设备数量的单位为台套；
- 5.单价50万元以下的设备不用填写；
- 6.本表只填写中央财政资金购置（试制）的设备。

序号	设备名称	设备分类	功能和技术指标	单价	数量	金额	购置或试制单位	安置单位	购置设备类型	生产厂家及国别	规格型号	拟开放共享范围
	(1)	(2)	(3)	(4)	(5)	(6)	(7)	(8)	(9)	(10)	(11)	(12)
无记录												
单价50万元以上购置设备合计							/	/	/	/	/	/
单价50万元以上试制设备合计							/	/	/	/	/	/
累计							/	/	/	/	/	/



课题单位经费预算明细表

表B3 课题编号： 2025YFF1309301 课题名称： 喀斯特矿区适生植物的优选、生态配置与资源化利用 金额单位：万元

填表说明：1.单位类型分课题承担单位、课题参与单位； 2.组织机构代码指企事业单位国家标准代码，单位若已三证合一请填写单位统一社会信用代码，无组织机构代码的单位填写“000000000”。										
序号	单位名称	组织机构代码-统一社会信用代码		单位类型	任务分工	研究任务 负责人	合计	中央财政专项资金		其他来源 资金
								小计	其中：间接 费用	
	(1)	(2)	(3)	(4)	(5)	(6)	(7)	(8)	(9)	(10)
1	桂林理工大学	统一社会信用代码	12450000498671388Q	课题承担单位	任务2：适应不同微地貌的根系养护和快速定植方法；任务4：植物群落近自然演替过程的正向调控措施	王敦球、刘杰	140.00	46.00	9.00	94.00
2	华南农业大学	统一社会信用代码	124400004554165634	课题参与单位	任务1：耐酸耐重金属先锋植物的选育；任务5：修复植物的资源化利用技术	程六龙、杨旭	100.00	34.00	8.00	66.00
3	广西科学院	统一社会信用代码	12450000498505056J	课题参与单位	任务3：植物生态配置模式优化及初始群落的构建	孙冬婧	50.00	17.00	5.00	33.00
累计							290.00	97.00	22.00	193.00



附件 2

其他来源资金承诺书

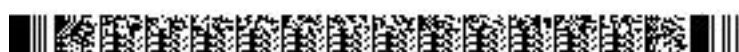
根据生态环境部《关于发布国家重点研发计划“大气与土壤、地下水污染综合治理”等 3 个重点专项 2025 年度定向项目申报指南的通知》，2025 年度定向指南拟围绕广西喀斯特矿区重金属污染的植物固定与生态修复技术与示范启动 1 个项目，采取定向择优方式支持，安排中央财政经费预算不超过 700 万元。由广西壮族自治区科学技术厅作为项目推荐单位组织申报，推荐 1 个项目，鼓励产学研联合申报。广西财政经费与中央财政经费比例为 2:1。据此，广西壮族自治区科学技术厅承诺按照申报指南通知要求，为“广西喀斯特矿区重金属污染的植物固定与生态修复技术与示范”项目，按照 2:1 进行配套，提供不超过 1400 万元的资金，资金来源为地方财政资金。

资金主要用于：设备费、业务费、劳务费及绩效支出等其他费用。

特此证明！

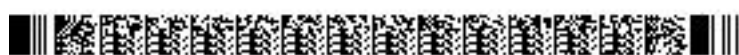
出资单位（公章）：

2025 年 8 月 20 日



十一、相关附件

1. 乙方与参加单位有关协议（须加盖乙方与参加单位公章、法人签字签章；协议文件须扫描上传。如无参加单位，则不填）；
2. 申报指南规定的其他附件。



课题实施合作协议

甲方：桂林理工大学

乙方：华南农业大学

为顺利实施和有效完成国家重点研发计划重点专项“典型脆弱生态系统保护与修复”重点专项 2025 年度项目“广西喀斯特矿区重金属污染的植物固定与生态修复技术与示范（2025YFF1309300）”，甲乙双方达成如下协议：

（1）工作分工：甲方是课题主持单位，全面负责课题一“喀斯特矿区适生植物的优选、生态配置与资源化利用（2025YFF1309301）”组织和实施。乙方负责课题一子课题 1“耐酸耐重金属先锋植物的选育”的研发工作，需要根据批复的项目和课题任务书有关内容，按时全面完成各项研究任务。

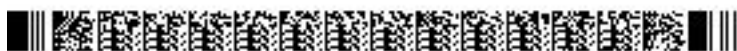
（2）经费分配：课题一总经费为 290.00 万，其中国拨经费 97.00 万，地方财政资金 193.00 万，其中子课题 1 经费 50.00 万元，其中国拨经费 17.00 万，地方财政资金 33.00 万。以实际批准经费额度为准，按同比例进行核减与调整。

（3）考核指标：乙方完成子课题 1“耐酸耐重金属先锋植物的选育”的研发工作：优选 6 种适合于示范区种植的耐酸耐重金属的快速定植和深根系植物，耐受 $\text{pH} \leq 5$ ，成活率 $> 90\%$ ，发表研究论文 1 篇。

（5）课题执行期间，乙方接受甲方的管理，及时向甲方汇报任务进展情况，并提交相关报告。

（6）课题执行期间产生的知识产权，按照各单位贡献大小排名。

（7）协议一式 8 份，提交专业机构 2 份，项目牵头单位 2 份，课题牵头单位保留 2 份，参与单位 2 份，具有同等法律效力。以下无正文，转签章页





甲方（单位签章）

法定代表人：

课题负责人：

时间：2025 年 10 月 29 日

王江华
王江华



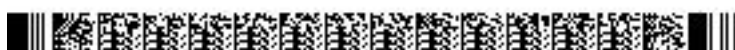
乙方（单位签章）

法定代表人：

子课题负责人：

时间：2025 年 10 月 31 日

薛旭



本页无正文，为签章页

（课题/任务牵头单位签章页）

项目依托单位（公章）：

中国科学院地理科学与资源研究所

法定代表人（签章）：

孙伟

项目负责人（签字）：

2025.8.22



合作单位（公章）：
华南农业大学

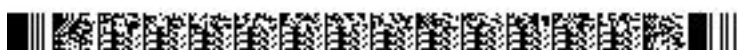
法定代表人（签章）：

薛红已

课题/任务负责人（签字）：

2025.8.25

李峰



科学技术部文件

国科发基〔2025〕127号

科技部关于科技基础资源调查专项 2025年度第二批工作任务 立项的通知

各有关部门：

科技基础资源调查专项 2025 年度第二批工作任务立项评审工作已完成。根据专家评审结果，经研究，决定批准“生物结构科学数据的整编和数据库建设”等 23 个工作任务立项（见附件）。请各有关部门和承担单位加强管理，认真做好组织实施工作。

联系人及电话：梁哲，010-58881528

附件:科技基础资源调查专项 2025 年度第二批工作任务立项
清单



(此件不公开)

附件

科技基础资源调查专项 2025 年度第二批工作任务立项清单

任务编号	任务名称	任务牵头单位	推荐部门	任务负责人
2025FY100300	生物结构科学数据的整编和数据库建设	清华大学	教育部	张强锋
2025FY100400	南方典型酸化农区生物多样性与性状调查	中国农业科学院 农业资源与农业区划研究所	农业农村部	吴红慧
2025FY100500	我国中重度盐碱地微生物资源调查与菌种资源库构建	中国科学院 新疆生态与地理研究所	中国科学院	李文均
2025FY100600	“三北”地区抗逆灌木资源本底调查和资源收集	中国林业科学研究院 沙漠林业实验中心	国家林草局	张景波
2025FY100700	东北黑土区农田抗生素赋存与分布特征调查	中国科学院 沈阳应用生态研究所	中国科学院	李婷婷
2025FY100800	燕山—太行山动物多样性科学考察	中国科学院动物研究所	中国科学院	陈军
2025FY100900	长江珍稀濒危鱼类调查与生殖干细胞收集保藏	中国科学院水生生物研究所	中国科学院	曹宏
2025FY101000	海洋牧场生态系统要素基础调查	中国科学院海洋研究所	中国科学院	孙丽娜

抄送：国家科技基础条件平台中心。

科学技术部办公厅

2025 年 9 月 23 日印发

科技基础资源调查专项
子任务书

子任务名称： 南方酸化农区典型管理模式果菜茶园土壤生物多样性及性状调查

子任务编号： 2025FY100404

所属任务： 南方典型酸化农区生物多样性与性状调查

任务牵头承担单位： 中国农业科学院农业资源与农业区划研究所

子任务承担单位（盖章）： 南京农业大学

子任务负责人（签字）： 陈小云

执行期限： 2026年01月至2028年12月

中华人民共和国科学技术部制

2026 年 1 月 8 日



填写说明

一、子任务书甲方即任务牵头承担单位，乙方即子任务承担单位。

二、子任务书通过“国家科技计划管理信息系统公共服务平台”，按照系统提示在线填写。

三、子任务书中的单位名称，请按规范全称填写，并与单位公章一致。

四、子任务书要求提供乙方与所有参加单位的合作协议，需对原件进行扫描后在线提交。

五、子任务书中文字须用宋体小四号字填写。

六、凡不填写内容的栏目，请用“无”表示。

七、乙方完成子任务书的在线填写，提交甲方审核确认后，用A4纸在线打印、装订、签章。一式八份报任务牵头承担单位签章，其中报科技部三份，任务推荐部门、任务牵头承担单位、任务负责人、子任务承担单位和子任务负责人各留存一份。

八、涉密子任务请在“国家科技计划管理信息系统公共服务平台”下载任务书的电子版模板，按保密要求离线填写、报送。

九、《任务申报书》和《任务书》是本任务书填报的重要依据，任务书填报不得降低考核指标，不得自行对主要研究内容作大的调整。《任务申报书》、

《任务书》和本子任务书将共同作为子任务过程管理、验收和监督评估的重要依据。



子任务预算表

表B2 子任务编号：2025FY100404

子任务名称：南方酸化农区典型管理模式果菜茶园土壤生物多样性及性状调查

金额单位：万元

序号	预算科目名称	金额
	(1)	(2)
1	一、中央财政专项资金	300.00
2	（一）直接费用	240.00
3	1. 设备费	0.00
4	其中：购置设备费	0.00
5	2. 业务费	212.64
6	3. 劳务费	27.36
7	（二）间接费用（自动计算）	60.00
8	二、其他来源资金	0.00
9	三、合计	300.00

注：1. 间接费用无需编制预算说明；2. 绩效支出在间接费用中无比例限制。承担单位在统筹安排间接费用时，要处理好合理分摊间接成本和对科研人员激励的关系，绩效支出安排与科研人员在子任务工作中的实际贡献挂钩。



单位研究经费支出预算明细表

表B4 子任务编号：2025FY100404 子任务名称：南方酸化农区典型管理模式果菜茶园土壤生物多样性及性状调查 金额单位：万元

填表说明：1. 单位类型为子任务承担单位、子任务参与单位。 2. 组织机构代码指企事业单位国家标准代码，单位若已三证合一请填写单位统一社会信用代码，无组织机构代码的单位填写“000000000”。										
序号	单位名称	组织机构代码-统一社会信用代码		单位类型	任务分工	研究任务负责人	合计	中央财政经费		其他来源资金
								小计	其中：间接费用	
	(1)	(2)	(3)	(4)	(5)	(6)	(7)	(8)	(9)	(10)
1	南京农业大学	统一社会信用代码	12100000466007562R	牵头单位	负责酸化农区典型管理模式果菜茶园蚯蚓和线虫调查	陈小云、胡正锟	150.00	150.00	30.00	0.00
2	中国农业科学院农业资源与农业区划研究所	统一社会信用代码	12100000400010670X	参与单位	鄂湘苏皖等省份酸化农区果菜茶园土壤微生物和原生物多样性调查	肖琼	50.00	50.00	10.00	0.00
3	华南农业大学	统一社会信用代码	124400004554165634	参与单位	粤闽赣浙等省份酸化农区果菜茶园土壤微生物和原生物多样性调查	杨旭	50.00	50.00	10.00	0.00
4	云南省农业科学院农业环境资源研究所	统一社会信用代码	12530000431202915P	参与单位	川渝黔滇桂琼等省份酸化农区果菜茶园土壤微生物和原生物多样性调查	张庆	50.00	50.00	10.00	0.00
累计							300.00	300.00	60.00	0.00



国家科技基础资源调查专项子任务

“南方酸化农区典型管理模式果菜茶园土壤生物多样性及性状调查”

(2025FY100404) 执行协议书

子任务依托单位（甲方）：南京农业大学

子任务参加单位（乙方）：华南农业大学

甲乙双方共同参加国家科技基础资源调查专项子任务“南方酸化农区典型管理模式果菜茶园土壤生物多样性及性状调查（2025FY100404）”的执行。经友好协商，达成如下协议：

1. 双方合作执行子任务，甲方主持并组织实施，乙方为参与单位之一。
2. 乙方完全理解和接受国家科技基础资源调查专项执行的一切规定和要求。乙方承诺对本单位承担的任务予以负责，保证支持本单位参加团队和参加人对该子任务工作实施，并按子任务实施方案与考核指标规定完成任务。
3. 甲方履行依托单位组织责任，并保证按科技部批复分配任务与经费。
4. 子任务执行过程中，甲乙双方将按照国家科技基础资源调查专项管理办法的要求和相关规定开展相关研究，并且严格履行相应义务。
5. 本协议未尽事宜，双方友好协商解决，不能解决的可上诉法院。
6. 本协议一式三份，盖章签字后生效。

子任务依托单位：南京农业大学

法定代表人（签字）：

子任务负责人（签字）：



2026年1月5日

子任务参加单位：华南农业大学

法定代表人（签字）：

子任务参加人（签字）：



2026年1月5日



任务书签署

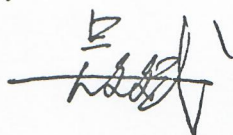
甲乙双方根据《国务院印发关于深化中央财政科技计划（专项、基金）管理改革方案的通知》（国发〔2014〕64号）、《国务院关于优化科研管理提升科研绩效若干措施的通知》（国发〔2018〕25号）、《国务院办公厅关于改革完善中央财政科研经费管理的若干意见》（国办发〔2021〕32号）、《科学技术活动违规行为处理暂行规定》（科学技术部令第19号）、《科技部财政部关于印发〈中央财政科技计划（专项、基金等）监督工作暂行规定〉的通知》（国科发政〔2015〕471号）、《科技部 自然科学基金委关于进一步压实国家科技计划（专项、基金等）任务承担单位科研作风学风和科研诚信主体责任的通知》（国科发监〔2020〕203号）等有关文件规定，以及有关法律、政策和管理要求，依据任务立项通知，签署本任务书。

同时，本单位和任务负责人**郑重承诺**：对本任务所有成果产出（包括但不限于新产品、新技术、标准、论文、专利等）的真实性、与任务的关联性等负责，将按要求落实科研作风学风和科研诚信主体责任；任务经费全部用于与本任务研究工作相关的支出，不截留、挪用、侵占，不用于与科学研究无关的支出；接受并积极配合相关部门的监督检查。如有违反，本单位和任务负责人以及相关成果产出者愿接受任务管理专业机构和相关部门做出的各项处理决定，包括但不限于终止任务执行、追回任务（子任务）经费，取消一定期限国家科技计划项目申报资格，记入科研诚信严重失信行为数据库以及主要负责人接受相应党纪政纪处理等。



任务牵头承担单位（甲方）：

法定代表人签字（签章）：





任务负责人签字（签章）：

吴红慧

2016年1月1日

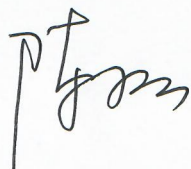
子任务承担单位（乙方）：

法定代表人签字（签章）：





子任务负责人签字（签章）：



2016年1月1日



受理编号: c22140500000450

项目编号: 2022A1515010662

文件编号: 粤基金字(2022)3号

广东省基础与应用基础研究基金项目 任务书

项目名称: 碳资源分配驱动植物-菌根共生体的重金属获取策略机理研究

项目类别: 广东省自然科学基金-面上项目

项目起止时间: 2022-01-01 至 2024-12-31

管理单位(甲方): 广东省基础与应用基础研究基金委员会

依托单位(乙方): 中山大学

通讯地址: 广东省广州市海珠区广州新港西路135号

邮政编码: 510275

单位电话: 020-84113181

项目负责人: 杨旭

联系电话: 020-84115962



(广东科技微信公众号)



(查看任务书信息)



(受理纸质材料二维码)

广东省基础与应用基础研究
基金委员会
二〇二〇年制

填写说明

一、项目任务书内容原则上要求与申报书相关内容保持一致，不得无故修改。

二、项目承担单位通过广东省科技业务管理阳光政务平台下载项目任务书，按要求完成签名盖章后提交至省科技厅受理窗口。

三、签名盖章说明。请分别在单位工作分工及经费分配情况页、人员信息页、签约各方页等地方按要求签字或盖章，签章不合规或错漏将不予受理。其中，人员信息页要求所有参与人员本人亲笔签名，代签或印章无效，漏签将不予受理。

四、本任务书自签字并加盖公章之日起生效，各方均应负本任务书的法律责任，不应受机构、人事变动影响。

2022A1515010662

一、主要研究内容和要达到的目标

丛枝菌根真菌 (AMF) 作为一种生物强化技术可以显著提高超富集植物对重金属污染土壤的修复效率。菌根真菌从土壤中吸收养分并与寄主植物进行交易以换取碳资源。而这部分碳源也是土壤微生物的部分资源基质, 直接影响着土壤其它微生物的生长。但植物-菌根共生体的碳资源分配如何影响共生体系对重金属的获取策略鲜有报道。因此, 探究碳资源分配如何驱动植物-菌根共生体的重金属获取具有鲜明的创新与特色。本项目将以镉超富集型东南景天与非超富集型东南景天作为研究对象, 揭示不同植物-菌根共生体系(超富集与非超富集植物)地上下部碳分配规律, 并探明根际与菌丝际分泌物组分变化以揭示超富集植物与非超富集植物对重金属镉获取策略的差异及相关机制。为深入了解植物-微生物互作提高土壤重金属污染的植物修复机制提供新的参考, 并进一步完善菌根在重金属污染土壤的植物修复中所扮演的重要角色。具体内容如下: 首先, 采用分室隔网盆栽培养系统, 使用 ^{14}C 同位素标记法, 分析中低镉浓度下, 不同植物-菌根共生体的光合碳资源在地上下部的分配规律, 并分析光照强度对植物-菌根共生体的碳分配影响。然后, 通过采用分室隔网盆栽培养系统, 分析超富集与非超富集植物的菌根际微界面分泌物组分特征, 并比较不同剖层土壤重金属的有效性, 以探讨不同植物-菌根共生体分泌物组成与土壤重金属活化之间的关系。最后, 通过 ^{13}C -DNA标记与宏基因组等分子手段, 探究不同类型植物-菌根共生体中根际与菌丝际利用植物光合产物的微生物群落特征与功能, 以揭示菌根-微生物的互作机制对菌根共生体重金属吸收的影响。

总体而言, 本项目拟揭示不同类型植物-菌根共生体地上部碳资源分配特征, 以探讨植物是否通过主动调控资源分配影响共生体系对重金属的获取策略; 同时, 揭示碳资源分配驱动根际与菌丝际分泌物变化及其根际与菌丝际微生物组成与功能差异, 为了解植物-菌根-其它微生物互作提高土壤重金属污染的植物修复提供重要的科学依据。

二、项目预期获得的科研成果及形式

论文及专著情况	国家统计源刊物以上刊物 发表论文（篇）		2		科技报告（篇）		1	
	其中被SCI/EI/ISTP收录 论文数（篇）		1		培养人才（人）			
	专著（册）				引进人才（人）			
专利情况(项)	发明专利		实用新型专利		外观设计专利		国外专利	
	申请	授权	申请	授权	申请	授权	申请	授权

三、项目进度和阶段目标

(一) 项目起止时间: 2022-01-01 至 2024-12-31		
(二) 项目实施进度及阶段主要目标:		
开始日期	结束日期	主要工作内容
2022-01-01	2022-12-31	在文献阅读基础上, 进一步细化试验方案, 并开展研究内容(1), 通过分室隔网培养系统与碳同位素标记的方法, 明确不同植物-菌根共生体的碳资源分配特征, 并分析植物-菌根共生体碳资源分配与重金属的吸收的关系。
2023-01-01	2023-12-31	在第一阶段的试验基础上, 继续开展研究内容(2), 通过分室隔网系统, 比较不同超富集植物与非超富集植物-菌根共生体的菌/根际微界面分泌物组分特征, 以探讨分泌物组成与土壤重金属活性之间的关系。总结数据, 并撰写、发表论文等。
2024-01-01	2024-12-31	在第二阶段的试验基础上, 开展研究内容(3), 通过多区室土培试验, 以 ¹³ C-DNA-SIP标记技术与宏基因组手段, 分析根际与菌丝际环境中利用了植物分泌物的活性微生物的种类和数量。最后总结研究结果, 撰写和发表论文; 并进行项目总结和撰写课题总结报告。 项目期间参加国内学术会议, 交流本项目的研究进展。

四、项目总经费及省基金委经费预算

1. 省基金委经费下达总额：（大写）壹拾万圆整；（小写）10万元；					
2. 省基金委经费年度下达计划：					
年度	2022 年	年	年	年	年
经费(万元)	10.00				
3. 总经费及省基金委经费开支预算计划：					
经费筹集情况：					(单位：万元)
省基金委经费	自筹资金				合计
	自有资金	贷款	地方政府投入	其它	
10.00	0.00	0.00	0.00	0.00	10.00
政府部门、境外资金及其他资金投入情况说明：	无				
与本项目相关的其他经费来源			(单位：万元)		
其他计划资助经费：			0.00		
单位配套经费：			0.00		
其他经费资助：			0.00		
其他经费来源合计：					

五、人员信息

项目负责人								
姓名	证件号码	年龄	性别	职称	学历	在项目中承担的任务	所在单位	签名
杨旭	440981199008191500	32	女	未取得	博士研究生	项目负责人	中山大学	杨旭

项目组主要成员								
姓名	证件号码	年龄	性别	职称	学历	在项目中承担的任务	所在单位	签名
储双双	429001198702247665	35	女	未取得	博士研究生	微生物互作机制实验	中山大学	储双双
孙丹	370682199502195023	27	女	未取得	硕士研究生	分子生物学机理分析	中山大学	孙丹
蔡煊	320106199406011615	28	男	未取得	硕士研究生	同位素标记分析	中山大学	蔡煊

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2022A1515010662 杨九

六、工作分工及经费分配

承担/参与单位名称 (盖章)	工作分工	总经费分摊 (万元)	省基金委经费分配 (万元)
中山大学	本单位负责本项目的申报、组织、实施和 结题。	10.00	10.00
2022年 5月 9日	合计	10.00	10.00

七、任务书条款

第一条 甲方与乙方根据《中华人民共和国民法典》及国家有关法规和规定，按照《广东省科学技术厅关于广东省基础与应用基础研究基金（省自然科学基金、联合基金等）项目管理的实施细则（试行）》《广东省省级科技计划项目验收结题工作规程（试行）》等规定，为顺利完成（2022）年碳资源分配驱动植物-菌根共生体的重金属获取策略机理研究专项项目（文件编号：粤基金字〔2022〕3号）经协商一致，特订立本任务书，作为甲乙双方在项目实施管理过程中共同遵守的依据。

第二条 甲方的权利义务：

1. 按任务书规定进行经费核拨的有关工作协调。
2. 根据甲方需要，在不影响乙方工作的前提下，定期或不定期对乙方项目的实施情况和经费使用情况进行检查或抽查。
3. 根据《广东省科研诚信管理办法(试行)》等规定对乙方进行科技计划信用管理。

第三条 乙方的权利义务：

1. 确保落实自筹经费及有关保障条件。
2. 按任务书规定，对甲方核拨的经费实行专款专用，单独列账，并随时配合甲方进行监督检查。
3. 经费使用按照广东省级财政科研项目经费使用等有关规定进行管理。
4. 项目依托单位应制定经费使用“负面清单+包干制”内部管理制度并报甲方备案。
5. 使用财政资金采购设备、原材料等，按照《广东省实施〈中华人民共和国招标投标法〉办法》有关规定，符合招标条件的须进行招标。
6. 项目任务书任务完成后，或任务书规定的任务、指标及经费投入等提前完成的，乙方可提出验收结题申请，并按甲方要求做好项目验收结题工作。
7. 若项目发生需要终止结题的情况，乙方须提出终止结题申请，并按甲方要求做好项目终止结题工作。
8. 在每年规定时间内向甲方如实提交上年度工作情况报告，报告内容包含上年度项目进展情况、经费决算和取得的成果等。
9. 按照国家和省有关规定，提交科技报告及其他材料。
10. 利用甲方的经费获得的研究成果，项目负责人和参与者应当注明获得“广东省基础与应用基础研究基金（英文：Guangdong Basic and Applied Basic Research Foundation）（项目编号）”资助或作有关说明。
11. 乙方要恪守科学道德准则，遵守科研活动规范，践行科研诚信要求，不得抄袭、剽窃他人科研成果或者伪造、篡改研究数据、研究结论；不得购买、代写、代投论文，虚构同行评议专家及评议意见；不得违反论文署名规范，擅自标注或虚假标注获得科技计划（专项、基金等）等资助；不得弄虚作假，骗取科技计划（专项、基金等）项目、科研经费以及奖励、荣誉等；不得有其他违背科研诚信要求的行为。
12. 确保本项目开展的研究工作符合我国科技伦理管理相关规定。

第四条 在履行本任务书的过程中，如出现广东省相关政策法规重大改变等不可抗力情况，甲方有权对所核拨经费的数量和时间进行相应调整。

第五条 在履行本任务书的过程中，当事人一方发现可能导致项目整体或部分失败的情形时，应及时通知另一方，并采取适当措施减少损失，没有及时通知并采取适当措施，致使损失扩大的，应当就扩大的损失承担责任。

第六条 本项目技术成果的归属、转让和实施技术成果所产生的经济利益的分享，除双方另有约定外，按国家和广东省有关法规执行。

第七条 根据项目具体情况，经双方另行协商订立的附加条款，作为本任务书正式内容的一部分，与本任务书具有同等效力。

第八条 本任务书一式三份，各份具有同等效力。甲、乙方及项目负责人各执一份，三方签字、盖章后即生效，有效期至项目结题后一年内。各方均应负任务书的法律责任，不应受机构、人事变动的影响。

第九条 乙方必须接受甲方聘请的本项目任务书监理单位的监督和管理。监理单位按照甲方赋予的权利对本项目任务书的履行进行审核、进度调查，对项目任务书变更、经费使用情况进行监督管理及组织项目验收。

说明：1. 本任务书中，凡是当事人约定无需填写的内容，应在空白处划（/）。

2. 委托代理人签订本任务书的，应出具合法、有效的委托书。

八、本任务书签约各方

管理单位（甲方）：

广东省基础与应用基础研究基金委员会（盖章）



法定代表人（或法人代理）：

（签章）

2022 年 04 月 21 日

依托单位（乙方）：中山大学

（盖章）

法定代表人（或法人代理）：高松

（签章）

联系人（项目主管）姓名：胡菁

（签章）

Email: yekq5@mail.sysu.edu.cn

电话：020-84110412 / 15919697929

开户单位名称：中山大学

开户银行名称：中国建设银行广州中山大学支行

开户银行帐号：44050143004609000001

2022 年 5 月 9 日
年 月 日

联系人（项目负责人）姓名：杨旭

（签名）

Email: yangx375@mail.sysu.edu.cn

电话：020-84115962

2022 年 4 月 24 日

受理编号: c25140500002662

项目编号: 2025A1515012774

文件编号: 粤基金字(2025)10号

广东省基础与应用基础研究基金项目 任务书

项目名称: AM真菌驱动蜈蚣草-玉米间作体系获取土壤砷的根系互作过程及其根际调控机制

项目类别: 广东省自然科学基金-面上项目

项目起止时间: 2025-01-01 至 2027-12-31

管理单位(甲方): 广东省基础与应用基础研究基金委员会

依托单位(乙方): 华南农业大学

通讯地址: 广东省广州市天河区五山路483号

邮政编码: 510642

单位电话: 020-85283435

项目负责人: 杨旭

联系电话: 020-84115962



(广东科技微信公众号)



(查看任务书信息)



(受理纸质材料二维码)

广东省基础与应用基础研究
基金委员会
二〇二〇年制

填写说明

一、项目任务书内容原则上要求与申报书相关内容保持一致，不得无故修改。

二、项目承担单位通过广东省科技业务管理阳光政务平台下载项目任务书，按要求完成签名盖章后扫描上传到广东省科技业务管理阳光政务平台。

三、签名盖章说明。请分别在单位工作分工及经费分配情况页、人员信息页、签约各方页等地方按要求签字或盖章，签章不合规或错漏将不予受理。其中，人员信息页要求所有参与人员本人亲笔签名，代签或印章无效，漏签将不予受理。

四、本任务书自签字并加盖公章之日起生效，各方均应负本任务书的法律责任，不应受机构、人事变动影响。

五、根据《广东省科学技术厅广东省财政厅关于深入推进省基础与应用基础研究基金项目经费使用“负面清单+包干制”改革试点工作的通知》（粤科规范字〔2022〕2号），2022年度及以后立项资助的全部省基金项目（包括省自然科学基金、省市联合基金、省企联合基金项目等）均适用“负面清单+包干制”，项目提交申请书和任务书时无需编制费用明细科目预算。

一、主要研究内容和要达到的目标

砷(As)污染是全球性的环境问题，我国As污染土壤广泛。针对我国人多耕地少的情况，利用As超富集植物与农作物间作进行As污染土壤修复是一种有效实现“边生产边修复”As污染土壤的绿色治理策略。目前，丛枝菌根(AM)真菌作为一种生物强化技术用于增强超富集植物间作体系对重金属污染土壤的修复效率。虽然当前对As超富集植物与农作物间作体系修复As污染土壤的效果有了一定的宏观认识，但针对于AM真菌如何驱动蜈蚣草-农作物间作体系的根系交互效应及其根际互惠策略鲜有报道。因此，探究AM真菌如何驱动As超富集植物-农作物间作体系对土壤As的界面活化与分配策略具有鲜明的创新与特色。本项目将以As超富集植物蜈蚣草-AM真菌-玉米复合体系为研究对象，揭示AM真菌介导蜈蚣草与间作玉米的根系生长空间分布规律与交互特征，探明间作体系根际分泌物组分变化以揭示两种间作植物对土壤As的根际活化与分配机制；并剖析AM真菌驱动间作体系中招募有关As吸收转化的土壤微生物群落种类和功能特点。本项目的顺利实施为深入了解AM真菌驱动间作体系增强土壤As污染的植物修复机制提供科学思路。本项目将系统探究AM真菌驱动As超富集植物与农作物的根系互作过程及其根际调控机制。具体内容如下：首先，通过根箱试验，采用根系原位扫描跟踪(Winrhizo)与根系网格原位取样等手段，细化间作蜈蚣草与玉米在生长过程中根系的分布过程，并重点关注两种植物是否存在根系重叠交互的特征，从时间与空间上探明间作体系中蜈蚣草与玉米的根系互作过程。其次，通过平面光极原位pH监测、LA-ICP-MS等原位界面原位研究手段，结合As形态变化与根际耗竭特征，探明AM真菌介导间作体系根际As活化与分配特点；通过非靶向代谢组分析方法，定量蜈蚣草-玉米间作体系中根际化学过程。最后，通过盆栽试验，采用高通量测序与宏基因组技术手段，分别探明蜈蚣草与玉米根际微生物组成与种类差异，并重点关注与土壤As转化有关微生物的功能基因变化，以剖析AM真菌驱动间作植物招募与选择微生物的作用机制。总体而言，本项目将揭示AM真菌驱动As超富集植物蜈蚣草与间作玉米根系互作过程，并通过其根际化学特征探究间作植物对于土壤As活化与分配的策略，同时揭示该体系对土壤微生物招募的选择机制与功能差异，为AM真菌如何增强超富集植物-农作物间作体系修复As污染土壤的根土界面互作机制提供理论依据，并为实现重金属污染土壤“边生产边修复边增效”的绿色治理目标提供参考思路。

二、项目预期获得的研究成果及形式

论文及专著情况	国家统计源刊物以上刊物 发表论文（篇）		2		科技报告（篇）		1	
	其中被SCI/EI/ISTP收录 论文数（篇）		1		培养人才（人）			
	专著（册）				引进人才（人）			
专利情况(项)	发明专利		实用新型专利		外观设计专利		国外专利	
	申请	授权	申请	授权	申请	授权	申请	授权

三、项目进度和阶段目标

(一) 项目起止时间: 2025-01-01 至 2027-12-31		
(二) 项目实施进度及阶段主要目标:		
开始日期	结束日期	主要工作内容
2025-01-01	2025-12-31	在文献阅读基础上, 进一步细化试验方案, 并开展研究内容(1), 通过根箱试验, 采用根系原位扫描跟踪(Winrhizo)与根系网格原位取样等手段, 分析间作植物根系空间分布与折叠交互的特征, 从时间与空间上探明间作体系中蜈蚣草与玉米的根系互作过程。
2026-01-01	2026-12-31	在第一阶段的试验基础上, 继续开展研究内容(2), 通过根盒培养系统, 通过平面光极原位pH监测、LA-ICP-MS等原位界面原位研究手段, 探明AM真菌介导间作体系根际As活化与分配特点。通过非靶向代谢组分析方法, 分析蜈蚣草-玉米间作体系中根际化学过程。期间总结实验结果, 分析数据, 并撰写论文等。
2027-01-01	2027-12-31	在第二阶段的试验基础上, 开展研究内容(3), 通过盆栽试验, 采用高通量测序、宏基因组与定量PCR手段, 探明蜈蚣草与玉米根际微生物组成与种类差异, 并重点关注与土壤As转化有关微生物的功能变化, 以剖析AM真菌驱动间作植物招募与选择微生物的作用机制。最后总结研究结果, 撰写和发表论文; 进行项目总结和撰写课题总结报告。 项目期间参加国内外学术会议, 交流本项目的研究进展。

四、项目总经费及省基金委经费预算

1. 省基金委经费下达总额： （大写）壹拾万圆整；（小写 ）10万元；					
2. 省基金委经费年度下达计划：					
年度	2025 年	年	年	年	年
经费(万元)	10.00				

五、人员信息

项目负责人								
姓名	证件号码	年龄	性别	职称	学历	在项目中承担的任务	所在单位	签名
杨旭	440981199008191500	35	女	副教授	博士研究生	项目负责人	华南农业大学	杨旭

项目组主要成员								
姓名	证件号码	年龄	性别	职称	学历	在项目中承担的任务	所在单位	签名
吴博涵	230422199306061329	32	女	助理研究员	博士研究生	根际强化机制探究	华南农业大学	吴博涵
姜彦岐	150429199705110057	28	男	未取得	硕士研究生	根际微生物招募机制研究	华南农业大学	姜彦岐
蒋卓民	431124200105130316	24	男	未取得	本科	间作根系分布及其参数测定	华南农业大学	蒋卓民

六、工作分工及财政经费分配

承担/参与单位名称 (盖章)	工作分工	省级财政科技资金分配 (万元)
华南农业大学	本单位负责本项目的申报、组织、实施和结题。	10.00
	合计	10.00

七、任务书条款

第一条 甲方与乙方根据《中华人民共和国民法典》及国家有关法规和规定，按照《广东省自然科学基金及联合基金项目管理实施细则》（粤科规范字〔2024〕5号）《省级科技计划项目任务书管理细则》（粤科规范字〔2022〕8号）等规定，为顺利完成（2025）年AM真菌驱动蜈蚣草-玉米间作体系获取土壤砷的根系互作过程及其根际调控机制专项项目（项目编号：2025A1515012774）经协商一致，特订立本任务书，作为甲乙双方在项目实施管理过程中共同遵守的依据。

第二条 甲方的权利义务：1. 按任务书规定进行经费核拨的有关工作协调。2. 根据甲方需要，在不影响乙方工作的前提下，定期或不定期对乙方项目的实施情况和经费使用情况进行检查或抽查。3. 根据《广东省科学技术厅科技计划项目科研诚信管理办法》（粤科规范字〔2024〕2号）《广东省基础与应用基础研究基金项目科研不端行为调查处理实施细则（试行）》（粤科规范字〔2023〕1号）等规定对乙方进行科技计划信用管理。

第三条 乙方的权利义务：1. 确保落实自筹经费及有关保障条件。2. 按任务书规定，对甲方核拨的经费实行专款专用，单独列账，并随时配合甲方进行监督检查。3. 经费使用按照广东省级财政科研项目经费使用及省基金项目经费使用“负面清单+包干制”等有关规定进行管理。4. 项目依托单位应制定经费使用“负面清单+包干制”内部管理制度并报甲方备案。5. 使用财政资金采购设备、原材料等，按照《广东省实施〈中华人民共和国招标投标法〉办法》有关规定，符合招标条件的须进行招标。6. 项目任务书任务完成后，或任务书规定的任务、指标及经费投入等提前完成的，乙方可提出验收结题申请，并按甲方要求做好项目验收结题工作。7. 若项目发生需要终止结题的情况，乙方须提出终止结题申请，并按甲方要求做好项目终止结题工作。8. 在每年规定时间内向甲方如实提交上年度工作情况报告，报告内容包含上年度项目进展情况、经费决算和取得的成果等。9. 按照国家和省有关规定，提交科技报告及其他材料。10. 利用甲方的经费获得的研究成果，项目负责人和参与者应当注明获得“广东省基础与应用基础研究基金（英文：Guangdong Basic and Applied Basic Research Foundation）（项目编号）”资助或作有关说明。11. 乙方要恪守科学道德准则，遵守科研活动规范，践行科研诚信要求，不得抄袭、剽窃他人科研成果或者伪造、篡改研究数据、研究结论；不得购买、代写、代投论文，虚构同行评议专家及评议意见；不得违反论文署名规范，擅自标注或虚假标注获得科技计划（专项、基金等）等资助；不得弄虚作假，骗取科技计划（专项、基金等）项目、科研经费以及奖励、荣誉等；不得有其他违背科研诚信要求的行为。12. 确保本项目开展的研究工作符合我国科技伦理管理相关规定。

第四条 在履行本任务书的过程中，如出现广东省相关政策法规重大改变等不可抗力情况，甲方有权对所核拨经费的数量和时间进行相应调整。

第五条 在履行本任务书的过程中，当事人一方发现可能导致项目整体或部分失败的情形时，应及时通知另一方，并采取适当措施减少损失，没有及时通知并采取适当措施，致使损失扩大的，应当就扩大的损失承担责任。

第六条 本项目技术成果的归属、转让和实施技术成果所产生的经济利益的分享，除双方另有约定外，按国家和广东省有关法规执行。

第七条 根据项目具体情况，经双方另行协商订立的附加条款，作为本任务书正式内容的一部分，与本任务书具有同等效力。

第八条 本任务书一式三份，各份具有同等效力。甲、乙方及项目负责人各执一份，三方签字、盖章后即生效，有效期至项目结题后一年内。各方均应负任务书的法律责任，不应受机构、人事变动的影响。

第九条 乙方必须接受甲方聘请的本项目任务书监理单位的监督和管理。监理单位按照甲方赋予的权利对本项目任务书的履行进行审核、进度调查，对项目任务书变更、经费使用情况进行监督管理及组织项目验收。

说明：1. 本任务书中，凡是当事人约定无需填写的内容，应在空白处划（/）。

2. 委托代理人签订本任务书的，应出具合法、有效的委托书。

八、本任务书签约各方

管理单位（甲方）：广东省基础与应用基础研究基金委员会（盖章）

法定代表人（或法人代理）：曾路（签章）



2025 年 03 月 21 日

依托单位（乙方）：华南农业大学（盖章）

法定代表人（或法人代理）：薛红卫（签章）

联系人（项目主管）姓名：夏杰（签章）

Email: kjcgxk@scau.edu.cn

电话：020-85283435 / 13711345768



开户单位名称：华南农业大学

开户银行名称：广东广州工行五山支行

开户银行账号：3602002609000310520

2025 年 4 月 9 日

联系人（项目负责人）姓名：杨旭（签名）

Email: yangxu@scau.edu.cn

电话：020-84115962

杨旭

2025 年 3 月 27 日



资助证书

中山大学 杨旭 （全国博管会编号 279814）获得中国博士后科学基金
第69批面上资助二等。 资助编号：2021M693665。

特颁此证。

The certificate certifies its holder is awarded the fellowship of China Postdoctoral
Science Foundation .

中国博士后科学基金会
2021 年 06 月 09 日



受理编号：SL2024A04J01896

广州市科技计划项目
申报书

项目名称：	菌根驱动超富集植物获取土壤镉的微生物调控机制
申报单位：	华南农业大学
项目负责人：	杨旭
计划类别：	基础 research 计划
专题名称：	2025年度基础与应用基础研究专题
支持方向：	青年博士“启航”项目
组织单位：	华南农业大学
起止时间：	2025-01-01 至 2026-12-31
主管处室：	引进智力管理处（科技人才处）

广州市科学技术局制

二〇二四年

填写说明

一、请申报单位认真阅读指南，所申报的项目研究内容须对应指南、符合指南的要求。

二、项目名称应清晰、准确反映研究内容，项目名称不宜宽泛，只能由中文、英文字符组成，不超过50中文字。

三、本申报书通过“广州科技大脑”在线填写、报送，不需要线下提交纸质材料。

四、申报书中的单位名称，请按规范全称填写，并与单位公章一致。

五、涉密项目请在“广州科技大脑”下载申报书的电子版模板，按保密要求离线填写、报送。

六、本申报书中凡是无需填写的内容，应在空白处划“/”，或用“无”表示。

七、申报书内容须按照项目申报书据实填写，要遵循实事求是原则，无需凑够字数。

一、基本信息

项目 基本 信息	项目名称	菌根驱动超富集植物获取土壤镉的微生物调控机制		
	学科领域1	生命综合处-生态学-污染生态学-污染生态学		
	指南发布日	2024年4月15日		
	申请市级财政 金额	5万元	研究期限	2025年1月1日- 2026年12月31日
项目 摘要	<p>丛枝菌根（AM）真菌与超富集植物共生有效提高植物的修复效率。但目前有关AM真菌驱动超富集植物与菌根际微生物的互作机制知之甚少。基于本人前期基础，本项目将围绕Cd超富集植物（龙葵与东南景天），分析其获取Cd与磷元素的特征，通过代谢组学、宏基因组与DNA-SIP等技术，揭示两种超富集植物代谢产物差异及其菌根际微生物的结构特征、功能变化。本项目为AMF-细菌互作调控超富集植物吸收重金属提供了科学思路。</p>			

二、申报单位情况

项目承担单位	单位名称	华南农业大学	统一社会信用代码	124400004554165634
	注册时间	1952-01-01	单位类型	高等院校
	注册地址	广东省广州市天河区五山路483号		
	办公地址	广东省广州市天河区五山路483号		
	联系人	姓名	倪慧群	
		手机号码	13711345768	
		电子邮箱	kjcgxk@scau.edu.cn	
	开户银行	广东广州工行五山支行		
	开户户名	华南农业大学		
银行账号	3602002609000310520			

三、项目负责人信息

姓名	杨旭	证件类型	身份证
证件号码	440981199008191500	性别	女
出生年月	1990-08-19	民族	汉族
国籍	中国	学历	博士研究生
学位	博士	学位授予国家 (或地区)	中国
职务	无	职称	中级
所学专业	生态学	手机号码	18811845469
办公电话	020-85281822	电子邮箱	yangxu@scau.edu.cn

四、项目经费信息

本项目总投入：¥（5）万元，其中，市财政科技经费：¥（5）万元，自筹经费：¥（0）万元。

1. 经费下达计划			
资金来源	小计	市财政科技经费	自筹经费
2025	5	5	0
总计	5	5	0

（单位：万元）

注：本专题纳入“包干制”，市财政科技经费按市科技计划项目经费“包干制”相关规定执行。

审核通过

五、预期代表性成果

项目负责人在项目实施期内，以该项目作为资助项目获得以下5种情形之一且经费使用符合规定的，由组织单位审核后通过验收。

（一）项目实施期内，以第一作者/通讯作者发表论文1篇或以上（须标注资助项目编号）；

（二）项目实施期内，以第一完成人申请或授权专利、软件著作权1项或以上；

（三）项目实施期内，获省级以上科技计划项目或人才项目支持1项或以上；

（四）项目实施期内，获省级以上科技奖励（含列入获奖团队成员名单）1项或以上；

（五）项目实施期内，获得职称晋升。

审核通过

六、承诺函

申请人:	杨旭
承担申报单位:	华南农业大学
项目名称:	菌根驱动超富集植物获取土壤镉的微生物调控机制
专题方向:	2025年度基础与应用基础研究专题-青年博士“启航”项目
<p>申请人承诺:</p> <p>本人根据项目申报指南的要求自愿提交项目（课题）申报书，在此郑重承诺：严格遵守《关于进一步加强科研诚信建设的若干意见》《关于进一步弘扬科学家精神 加强作风和学风建设的意见》等有关规定，杜绝《科学技术活动违规行为处理暂行规定》（科学技术部令第19号）所列违规行为，所申报材料和相关内容真实有效，不存在违背科研诚信要求的行为；已按要求落实了科研作风学风和科研诚信主体责任；不得以任何形式实施请托行为，申报材料符合《中华人民共和国保守国家秘密法》和《科学技术保密规定》等相关法律法规，符合指南各项申报要求；在参与广州市科技计划项目申报、评审和实施全过程中，恪守职业规范和科学道德，遵守评审规则和工作纪律，杜绝以下行为：</p> <p>（一）抄袭、剽窃他人科研成果或者伪造、篡改研究数据、研究结论或实施其他侵犯他人知识产权的行为；</p> <p>（二）购买、代写、代投论文，虚构同行评议专家及评议意见；</p> <p>（三）违反论文署名规范，擅自标注或虚假标注获得科技计划等资助；</p> <p>（四）违反科研伦理规范；</p> <p>（五）弄虚作假，骗取科技计划项目、科研经费以及奖励、荣誉等；</p> <p>（六）在申报书中以高指标通过评审，在任务书签订时故意篡改降低任务书中相应指标；</p> <p>（七）以任何形式打听尚未公布的评审专家名单及其他评审过程中的保密信息；</p> <p>（八）本人或委托他人通过各种方式及各种途径联系有关专家进行请托、游说，违规到评审会议驻地游说评审专家和工作人员、询问评审或尚未正</p>	

式向社会公布的信息等干扰评审或可能影响评审公正性的活动；

（九）向评审工作人员、评审专家等提供任何形式的礼品、礼金、有价证券、支付凭证、商业预付卡、电子红包，或提供宴请、旅游、娱乐健身等任何可能影响评审公正性的活动；

（十）其它违反财经纪律和相关管理规定的行为。

如有违反，本人愿接受项目管理机构和相关部门做出的各项处理决定，包括但不限于取消项目（课题）承担资格，追回项目（课题）经费，向社会通报违规情况，取消一定期限广州市科技计划项目申报资格，记入科研诚信严重失信行为数据库以及接受相应的党纪政纪处理等。

签字：杨旭

日期：2024年07月02日

审核通过

承担单位承诺：

本单位根据项目申报指南的任务需求，严格履行承担单位职责，自愿审核提交申报书，**在此郑重承诺：**

严格遵守《关于进一步加强科研诚信建设的若干意见》《关于进一步弘扬科学家精神 加强作风和学风建设的意见》等有关规定和其它科研诚信要求的行为，已按要求落实了科研作风学风和科研诚信主体责任；不以任何形式实施请托行为，申报材料符合《中华人民共和国保守国家秘密法》和《科学技术保密规定》等相关法律法规，符合指南各项申报要求；在参与项目申报和评审活动全过程中，遵守有关评审规则和工作纪律，杜绝以下行为：

（一）采取贿赂或变相贿赂、造假、剽窃、故意重复申报等不正当手段获取科技计划项目承担资格；

（二）以任何形式探听未公开的评审专家名单及其他评审过程中的保密信息；

（三）组织或协助项目团队向评审工作人员、评审专家等提供任何形式的礼品、礼金、有价证券、支付凭证、商业预付卡、电子红包等；宴请评审组织者、评审专家，或向评审组织者、评审专家提供旅游、娱乐健身等可能影响评审公正性的活动；

（四）包庇、纵容项目团队虚假申报项目，甚至骗取国家科技计划项目；

（五）包庇、纵容项目团队，甚至帮助项目团队采取“打招呼”等方式，影响评审公正；

（六）在正式申报书中以高指标通过评审，在任务书签订时故意篡改降低任务书中相应指标；

（七）其它违反财经纪律和相关管理规定的行为。

如有违反，本单位愿接受项目管理机构和相关部门做出的各项处理决定，包括但不限于停拨或核减经费，追回项目（课题）经费，取消一定期限广州市科技计划项目申报资格，记入科研诚信严重失信行为数据库等。

承担单位：华南农业大学

日期：2024年07月02日

七、单位审核

承担单位意见：

同意申报。

日期：2024年07月05日

组织单位意见：

通过

日期：2024年07月11日

审核通过



项目批准号	42230707
申请代码	D0711
归口管理部门	
依托单位代码	51064208A0499-0932



422307071009788

国家自然科学基金 资助项目计划书 (预算制项目)

资助类别: 重点项目

亚类说明:

附注说明: 人类活动与环境

项目名称: 镉纳米胶体在稻田-水稻系统中的形成转化机制与生物有效性

直接费用: 272万元 执行年限: 2023.01-2027.12

负责人: 仇荣亮

通讯地址: 广东省广州市天河区五山华南农业大学

邮政编码: 510642 电话: 020-85285282

电子邮件: qiurl@scau.edu.cn

依托单位: 华南农业大学

联系人: 唐家林 电话: 020-85280070

填表日期: 2022年09月14日

国家自然科学基金委员会制

Version: 1.009.788



国家自然科学基金资助项目计划书填报说明 （预算制项目）

- 一、项目负责人收到《国家自然科学基金资助项目批准通知》（以下简称《批准通知》）后，请认真阅读本填报说明，参照国家自然科学基金相关项目管理办​​法和新修订的《国家自然科学基金资助项目资金管理办法》（以下简称《资金管理办法》，请查阅国家自然科学基金委员会官方网站首页“政策法规”栏目），按《批准通知》的要求认真填写和提交《国家自然科学基金资助项目计划书》（以下简称《计划书》）。
- 二、填写《计划书》时要科学严谨、实事求是、表述清晰、准确。《计划书》经国家自然科学基金委员会相关项目管理部门审核批准后，将作为项目研究计划执行、检查和验收的依据。
- 三、《计划书》各部分填写要求如下：
 - （一）简表：由系统自动生成。
 - （二）摘要及关键词：各类获资助项目都应当填写中、英文摘要及关键词。
 - （三）项目组主要成员：计划书中列出姓名的项目组主要成员由系统自动生成，与申请书原成员保持一致，不可随意调整。如果《批准通知》所附“项目评审意见及修改意见表”中“修改意见”栏目有调整项目组成员相关要求的，待项目开始执行后，按照项目成员变更程序另行办理。
 - （四）资金预算表：根据批准的项目资助额度，按规定调整项目预算，并按照《国家自然科学基金项目计划书预算表编制说明》填报资金预算表和预算说明书。
 - （五）正文：
 1. 面上项目、地区科学基金项目：如果《批准通知》所附“项目评审意见及修改意见表”中“修改意见”栏目没有修改要求的，只需选择“研究内容和研究目标按照申请书执行”即可；如果《批准通知》中上述栏目明确要求调整研究期限或研究内容等的，须选择“根据研究方案修改意见更改”并填报相关修改内容。
 2. 重点项目、重点国际（地区）合作研究项目、重大项目、国家重大科研仪器研制项目、原创探索计划项目：须选择“根据研究方案修改意见更改”，根据《批准通知》的要求填写研究（研制）内容，不得自行降低、更改研究目标（或仪器研制的技术性能与主要技术指标、验收技术指标等）或缩减研究（研制）内容。此外，还要突出以下几点：
 - （1）研究的难点和在实施过程中可能遇到的问题（或仪器研制风险），拟采用的研究（研制）方案和技术路线；
 - （2）项目主要参与者分工，合作研究单位（如有）之间的关系与分工，重大项目还需说明课题之间的关联；
 - （3）详细的年度研究（研制）计划。
 3. 创新研究群体项目：须选择“根据研究方案修改意见更改”，按下列提纲撰写：
 - （1）研究方向；

- (2) 结合国内外研究现状，说明研究工作的学术思想和科学意义（限两个页面）；
 - (3) 研究内容、研究方案及预期目标（限两个页面）；
 - (4) 年度研究计划；
 - (5) 研究队伍的组成情况。
4. 基础科学中心项目：须选择“根据研究方案修改意见更改”，根据《批准通知》的要求和现场考察专家组的意见和建议，进一步完善并细化研究计划，按下列提纲撰写：
- (1) 五年拟开展的研究工作（包括主要研究方向、关键科学问题与研究内容）；
 - (2) 研究方案（包括骨干成员之间的分工及合作方式、学科交叉融合研究计划等）；
 - (3) 年度研究计划；
 - (4) 五年预期目标和可能取得的重大突破等；
 - (5) 研究队伍的组成情况。
5. 对于其他类型项目，参照面上项目的方式进行选择和填写。



简表

项目负责人信息	姓 名	仇荣亮	性 别	男	出生年月	1967年11月	民 族	汉族
	学 位	博士			职 称	教授		
	是否在站博士后	否			电子邮件	qiurl@scau.edu.cn		
	电 话	020-85285282			个人网页			
	工 作 单 位	华南农业大学						
	所 在 院 系 所	资源环境学院						
依托单位信息	名 称	华南农业大学					代码	51064208A0499
	联 系 人	唐家林			电子邮件	kycjkh@scau.edu.cn		
	电 话	020-85280070			网站地址	http://kjc.scau.edu.cn/		
合作单位信息	单 位 名 称							
	中山大学							
项目基本信息	项 目 名 称	镉纳米胶体在稻田-水稻系统中的形成转化机制与生物有效性						
	资 助 类 别	重点项目				亚 类 说 明		
	附 注 说 明	人类活动与环境						
	申 请 代 码	D0711:污染物环境行为与效应						
	基 地 类 别							
	执 行 年 限	2023.01-2027.12						
	直 接 费 用	272万元						

项目组主要成员

编号	姓名	出生年月	性别	职称	学位	单位名称	电话	证件号码	项目分工	每年工作时间（月）
1	仇荣亮	1967.11	男	教授	博士	华南农业大学	020-85285282	320113196711114813	项目负责人	6
2	齐华	1989.09	女	教授	博士	华南农业大学	13431006696	130635198909050826	植物分子生物学	6
3	严博方	1988.09	男	无	博士	中山大学	020-39332743	210402198809090515	稳定同位素标记及原位分析	9
4	丁铿博	1991.08	男	无	博士	中山大学	020-39332758	445102199108230058	土壤金属胶体形成转化	9
5	张妙月	1987.11	女	副研究员	博士	中山大学	020-84113454	420582198711120022	土壤金属胶体释放迁移	6
6	赵春梅	1984.11	女	副教授	博士	中山大学	02033932746	410901198411080542	迁移-累积模型	6
7	柳婷	1988.10	女	无	博士	华南农业大学	15285959597	421023198810070044	植物元素吸收	9
8	杨旭	1990.08	女	无	博士	中山大学	18811845469	440981199008191500	植物根际作用	9
9	杨崇	1971.03	男	实验师	学士	华南农业大学	020-85280198	440106197103281931	质谱等谱学分析	6
10	温宏睿	1978.12	女	实验师	硕士	华南农业大学	020-85280198	532701197812270025	颗粒物分离表征	6
总人数		高级		中级		初级	博士后		博士生	硕士生
12		4		2			4		2	



国家自然科学基金预算制项目预算表

项目批准号：42230707

项目负责人：仇荣亮

金额单位：万元

序号	科目名称	金额
1	一、基金资助项目直接费用合计	272.0000
2	1、设备费	26.0000
3	其中：设备购置费	19.0000
4	2、业务费	176.0000
5	3、劳务费	70.0000
6	二、其他来源资金	0.0000
7	三、合计	272.0000

注：请按照项目研究实际需要合理填写各科目预算金额。

国家自然科学基金项目负责人、依托单位承诺书

国家自然科学基金项目负责人承诺书

本人郑重承诺：我接受国家自然科学基金的资助，严格遵守中共中央办公厅、国务院办公厅《关于进一步加强科研诚信建设的若干意见》《关于进一步弘扬科学家精神加强作风和学风建设的意见》《关于加强科技伦理治理的意见》等规定，及国家自然科学基金委员会关于资助项目管理、项目资金管理等各项规章，在《计划书》填写及项目执行过程中：

（一）按照《批准通知》《国家自然科学基金资助项目计划书填报说明》的要求填写《计划书》，未自行降低、更改目标任务或约定要求，或缩减研究（研制）内容；

（二）树立“红线”意识，严格履行科研合同义务，按照《计划书》负责实施本项目（批准号：42230707），切实保证研究工作时间，按时报送有关材料，及时报告重大情况变动，不违规将科研任务转包、分包他人，不以项目实施周期外或不相关成果充抵交差；

（三）遵守科研诚信、科技伦理规范和学术道德，认真开展研究工作，对资助项目发表的论著和取得的研究成果按规定进行标注，不在非本项目资助的成果或其他无关成果上标注本项目批准号，反对无实质学术贡献者“挂名”，不在成果署名、知识产权归属等方面侵占他人合法权益，并如实报告本人及项目组成员发生的违背科研诚信要求的任何行为；

（四）尊重科研规律，弘扬科学家精神，严谨求实，追求卓越，反对浮夸浮躁、投机取巧，不人为夸大学术或技术价值，不传播未经科学验证的现象和观点；

（五）将项目资金全部用于与本项目研究工作相关的支出，并结合科研活动需要，科学合理安排项目资金支出进度；

（六）做好项目组成员的教育和管理，确保遵守以上相关要求。

如违背上述承诺，本人愿接受国家自然科学基金委员会和相关部门做出的各项处理决定。

项目负责人（签字）：

2021年9月25日

依托单位科研管理部门：

科学研究所

负责人（签章）：

2021年9月30日

依托单位财务管理部门：

负责人（签章）：

2021年9月30日

国家自然科学基金项目依托单位承诺书

我单位同意承担上述国家自然科学基金项目，将保证项目负责人及其研究队伍的稳定和研究项目实施所需的条件，严格遵守国家自然科学基金委员会有关资助项目管理、项目资金管理、科研诚信管理和科技伦理管理等各项规定，并督促实施。

依托单位（公章）

2021年9月30日

国家自然科学基金资助项目签批审核表

科学处审查意见：

同意按计划执行

负责人（签章）：

2022 年 12 月 10 日

本栏目由自然科学基金委填写

科学部审查意见：

同意按计划执行

负责人（签章）：

2022 年 12 月 15 日



项目名称： 镉纳米胶体在稻田-水稻系统中的形成转化机制与生物有效性

资助类型： 重点项目/人类活动与环境

申请代码： D0711. 污染物环境行为与效应

国家自然科学基金项目申请人和参与者承诺书

为了维护国家自然科学基金项目评审公平、公正，共同营造风清气正的科研生态，本人**在此郑重承诺**：严格遵守《中华人民共和国科学技术进步法》《国家自然科学基金条例》《关于进一步加强科研诚信建设的若干意见》《关于进一步弘扬科学家精神加强作风和学风建设的意见》以及科技部、自然科学基金委关于科研诚信建设有关规定和要求；申请材料信息真实准确，不含任何涉密信息或敏感信息，不含任何违反法律法规或违反科研伦理规范的内容；在国家自然科学基金项目申请、评审和执行全过程中，恪守职业规范和科学道德，遵守评审规则和工作纪律，杜绝以下行为：

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- (二) 购买、代写申请书；购买、代写、代投论文，虚构同行评议专家及评议意见；购买实验数据；
- (三) 违反成果发表规范、署名规范、引用规范，擅自标注或虚假标注获得科技计划等资助；
- (四) 在项目申请书中以高指标通过评审，在项目计划书中故意篡改降低相应指标；
- (五) 以任何形式打听或散布尚未公布的评审专家名单及其他评审过程中的保密信息；
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- (七) 向工作人员、评审专家等提供任何形式的礼品、礼金、有价证券、支付凭证、商业预付卡、电子红包，或提供宴请、旅游、娱乐健身等任何可能影响评审公正性的活动；
- (八) 违反财经纪律和相关管理规定的行为；
- (九) 其他弄虚作假行为。

如违背上述承诺，本人愿接受国家自然科学基金委员会和相关部门做出的各项处理决定，包括但不限于撤销科学基金资助项目，追回项目资助经费，向社会通报违规情况，取消一定期限国家自然科学基金项目申请资格，记入科研诚信严重失信行为数据库以及接受相应的党纪政务处分等。

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3	丁铿博 / 中山大学 / 4*****8	丁铿博
4	张妙月 / 中山大学 / 4*****2	张妙月
5	赵春梅 / 中山大学 / 4*****2	赵春梅
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9	温宏睿 / 华南农业大学 / 5*****5	温宏睿



项目名称： 镉纳米胶体在稻田-水稻系统中的形成转化机制与生物有效性
资助类型： 重点项目/人类活动与环境
申请代码： D0711. 污染物环境行为与效应

国家自然科学基金项目申请单位承诺书

为了维护国家自然科学基金项目评审公平、公正，共同营造风清气正的科研生态，**本单位郑重承诺**：申请材料中不存在违背《中华人民共和国科学技术进步法》《国家自然科学基金条例》《关于进一步加强科研诚信建设的若干意见》《关于进一步弘扬科学家精神加强作风和学风建设的意见》以及科技部、自然科学基金委关于科研诚信建设有关规定和要求的情况；申请材料符合《中华人民共和国保守国家秘密法》和《科学技术保密规定》等有关法律法规和规章制度要求，不含任何涉密信息或敏感信息；申请材料不含任何违反法律法规或违反科研伦理规范的内容；申请人符合相应项目的申请资格；在项目申请和评审活动全过程中，遵守有关评审规则和工作纪律，杜绝以下行为：

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（三）支持、放任或对申请人/参与者抄袭、剽窃、重复申报、提供虚假信息（含身份和学术信息）等不当手段申报国家自然科学基金项目疏于管理；

（四）支持或协助申请人/参与者采取“打招呼”“围会”等方式影响科学基金项目评审；

（五）其他违反财经纪律和相关管理规定的行为。

如违上述承诺，本单位愿接受自然科学基金委和相关部门做出的各项处理决定，包括但不限于停拨或核减经费、追回项目已拨经费、取消本单位一定期限国家自然科学基金项目申请资格、记入科研诚信严重失信行为数据库以及主要责任人接受相应党纪政务处分等。

依托单位公章：

日期：2022年9月30日

合作研究单位公章：

日期：2022年9月27日

合作研究单位公章：

日期： 年 月 日

检索证明

根据委托人提供的论文材料, 委托人华南农业大学资源环境学院 杨旭(学科类型:自然科学) 8 篇论文收录情况如下表。

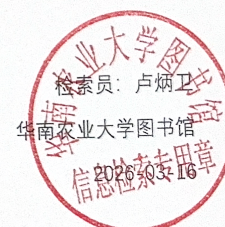
序号	论文名称	发表刊物及发表的年月卷期/页码等	作者排名	论文等级	作者文中单位	收录情况	影响因子	中科院大类分区
1	Upland rice intercropping with <i>Solanum nigrum</i> inoculated with arbuscular mycorrhizal fungi reduces grain Cd while promoting phytoremediation of Cd-contaminated soil	JOURNAL OF HAZARDOUS MATERIALS 出版年: 2021 出版日期: MAR 15 卷期: 406 页码: - 文献号: 124325 文献类型: Article	1	T2 类	华南农业大学	SCI	IF2-year=14.224 IF5-year=12.984 (2021)	环境科学与生态学 1 区 Top 期刊: 是 OA 期刊: 否 (2021)
2	Mycorrhizal fungi drive Cd and P allocation strategies for the co-planting system of hyperaccumulator <i>S. nigrum</i> and upland rice	ENVIRONMENTAL POLLUTION 出版年: 2025 出版日期: JUL 1 卷期: 376 页码: - 文献号: 126382 文献类型: Article	1	A 类	华南农业大学	SCI	IF2-year=7.3 IF5-year=8.1 (2025)	环境科学与生态学 2 区 Top 期刊: 否 OA 期刊: 否 (2025)
3	Salinity elevates Cd bioaccumulation of sea rice cultured under co-exposure of cadmium and salt	JOURNAL OF ENVIRONMENTAL SCIENCES 出版年: 2023 出版日期: APR 卷期: 126 页码: 602-611	1	A 类	中山大学	SCI	IF2-year=5.9 IF5-year=5.6 (2023)	环境科学与生态学 2 区 Top 期刊: 否 OA 期刊: 否 (2023)

		文献类型: Article						
4	Role of passivators for Cd alleviation in rice-water spinach intercropping system	ECOTOXICOLOGY AND ENVIRONMENTAL SAFETY 出版年: 2020 出版日期: DEC 1 卷期: 205 页码: - 文献号: 111321 文献类型: Article	1	A 类	华南农业大学	SCI	IF2-year=6.291 IF5-year=6.393 (2020)	环境科学与生态学 2 区 Top 期刊: 是 OA 期刊: 否 (2020)
5	Comparison of Cd subcellular distribution and Cd detoxification between low/high Cd-accumulative rice cultivars and sea rice	ECOTOXICOLOGY AND ENVIRONMENTAL SAFETY 出版年: 2019 出版日期: DEC 15 卷期: 185 页码: - 文献号: 109698 文献类型: Article	1	A 类	华南农业大学	SCI	IF2-year=4.872 IF5-year=4.967 (2019)	环境科学与生态学 2 区 Top 期刊: 是 OA 期刊: 否 (2019)
6	Effect of rainwater-borne hydrogen peroxide on manure-derived Cu and Zn speciation distribution and bioavailability in rice-soil system	ECOTOXICOLOGY AND ENVIRONMENTAL SAFETY 出版年: 2019 出版日期: AUG 15 卷期: 177 页码: 1-6 文献类型: Article	1	A 类	华南农业大学	SCI	IF2-year=4.872 IF5-year=4.967 (2019)	环境科学与生态学 2 区 Top 期刊: 是 OA 期刊: 否 (2019)

7	我国与欧美化肥重金属限量标准的比较和启示	植物营养与肥科学报 出版年: 2019 出版日期: 2019-01-07 16:42 卷期: 25 01 页码: - 文献号: 文献类型: 期刊论文	1	B 类	华南农业大学	CNKI	无	无
8	农用聚磷酸铵在土壤中的有效性研究进展及在农业上的应用	中国土壤与肥料 出版年: 2018 出版日期: 2018-06-10 卷期: 03 页码: - 文献号: 文献类型: 期刊论文	1	C 类	华南农业大学	北大核心	无	无

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根据委托人提供的论文材料，委托人华南农业大学资源环境学院 杨旭(学科类型:自然科学) 2 篇论文收录情况如下表。

序号	论文名称	发表刊物及发表的年月卷期/页码等	作者排名	论文等级	作者文中单位	收录情况	影响因子	中科院大类分区
1	Co-application of earthworms and arbuscular mycorrhizal fungi enhances arsenic tolerance of upland rice and improves soil health	JOURNAL OF ENVIRONMENTAL MANAGEMENT 出版年: 2025 出版日期: MAY 卷期: 381 页码: - 文献号: 125213 文献类型: Article	共同通讯作者	A 类	华南农业大学	SCI	IF2-year=8.4 IF5-year=8.6 (2024)	环境科学与生态学 2 区 Top 期刊: 是 OA 期刊: 否 标注: Mega-Journal (2025)
2	The potential of earthworms and arbuscular mycorrhizal fungi to enhance phytoremediation in heavy metal-contaminated soils: a review	MYCORRHIZA 出版年: 2025 出版日期: APR 24 卷期: 35 3 页码: - 文献号: 33 文献类型: Review	共同通讯作者	A 类	华南农业大学	SCI	IF2-year=3.8 IF5-year=3.7 (2024)	生物学 2 区 Top 期刊: 否 OA 期刊: 否 (2025)

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Upland rice intercropping with *Solanum nigrum* inoculated with arbuscular mycorrhizal fungi reduces grain Cd while promoting phytoremediation of Cd-contaminated soil

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ABSTRACT

Intercropping of hyperaccumulators with crops is a promising measure to enhance phytoremediation without impeding agricultural production. A Cd-hyperaccumulator, *Solanum nigrum* L. (*S. nigrum*), was intercropped with upland rice in a pot and rhizo-box experiment with Cd-contaminated soil to evaluate the combined effects of intercropping and arbuscular mycorrhizal fungi on plant growth and Cd accumulation. The results showed that, compared with monoculture, the combined treatments markedly decreased Cd concentration in rice parts, with the lowest Cd concentration in brown rice (reducing by 64.5%). The spatial distribution of root surface area and DTPA-Cd in the rhizo-box indicated competitive Cd uptake by neighbouring *S. nigrum*. Moreover, the combined treatments reduced *Nramp5* expression but increased *HMA3* levels in rice roots, leading to lower bioaccumulation and transfer coefficients. Additionally, fewer secreted organic acids and a higher rhizosphere pH were observed in rice. Conversely, the combined treatments promoted biomass, root length, root surface area, and decreased the rhizosphere pH in *S. nigrum*, thus increasing the Cd accumulation. Although the intercropping system with AMF inoculation notably reduced rice yield, the land-use efficiency was higher. These results provided insights into the role of AMF in the upland rice/*S. nigrum* system and demonstrated an alternative system for Cd phytoremediation.

1. Introduction

Agricultural farmlands contaminated with cadmium (Cd) is a serious public health concern worldwide due to its potential threat to human health (Wiggenhauser et al., 2016). Cd is one of the most concerning pollutants in China, with 7% of the land area impaired by Cd contamination (Wu et al., 2018). At present, three predominant measures are used to lower the Cd bioavailability in soil: isolation, removal, and stabilization (Ji et al., 2011). Compared to some physical and chemical methods, phytoremediation by plants (e.g., hyperaccumulator species) is the most promising because of its advantages of high efficiency, low cost, lack of secondary pollution, and in situ nature (Ma et al., 2020). However, large-scale application of hyperaccumulator plants is still limited because of the small biomass, slow growth of

hyperaccumulators, and low bioavailability of metals in soil (Sarwar et al., 2017).

It is well documented that intercropping has the advantages of enabling better light usage in plants, soil fertility, time, and space resources (Y. Tang et al., 2017). In recent years, it has been proposed to use the hyperaccumulators and the relatively low-accumulator crops in intercropping systems in combination to improve the remediation efficiency in agricultural practices (Hu et al., 2013; Tang et al., 2020). These intercropping systems can increase the biomass and the metal acquisition of hyperaccumulators, while decrease the metal uptake in the target plants, being a potential way to remediate the contaminated soil without impeding agricultural production (Xia et al., 2018; Luo et al., 2019). When intercropped with a hyperaccumulator, interlacing of the roots elevates the remediation of soil contaminated by heavy metals, thus

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reducing heavy metal acquisition by the target crops (Y. Tang et al., 2017). In our previous study, the cassava/peanut and the water spinach/rice intercropping systems notably indicate a yield advantage and reduce Cd content in the target crops (Zeng et al., 2019; Kang et al., 2020).

Solanum nigrum L. (*S. nigrum*), a Cd hyperaccumulator, has showed good prospects in remediating Cd-polluted soils in many studies (Wei et al., 2005; Yang et al., 2020). Some measures are taken to promote the remediation efficiency of *S. nigrum*, such as AMF (*Rhizophagus irregularis*) inoculation (G. Wang et al., 2020), changes to the electric conditions (Xu et al., 2020), or improvements to the soil nutrients (Yang et al., 2020), etc. *Funnelliformis mosseae*, one of the common AMF species, has been reported to promote metal bioavailability and facilitate root to shoot translocation of hyperaccumulators (Al Agely et al., 2005; Puna-miya et al., 2010). For example, after symbiotic with *Funnelliformis mosseae*, *S. nigrum* exhibits better growth and markedly increase Cd uptake with better metal-induced stress tolerance (Jiang et al., 2016). *S. nigrum* inoculated with AMF ameliorates growth by enhancement of productivity and nutrient acquisition and reduces pH to increase Cd bioavailability in the soil, thus increasing Cd uptake in the plant (G. Wang et al., 2020). However, to our knowledge, there have been few investigations conducted to study the remediation effects of *S. nigrum*-inoculated AMF in an intercropping system.

Rice (*Oryza sativa* L.) is the main staple crop in Asian countries, and the high Cd intake from rice plants potentially leads to serious impacts on public health (Chen et al., 2019). Water scarcity hinders the sustainability of agricultural systems in different parts of Asia, and rice cultivation expends more than half of the irrigation water in agricultural production (Dawe, 2005; Hu et al., 2015). Therefore, the cultivation of upland rice is considered as an effective way to increase water use efficiency for food production (Khaliq et al., 2019). Many investigations have demonstrated the major transport processes for rice Cd accumulation: (1) Cd uptake by root, (2) root-to-shoot translocation by xylem flow, (3) redirection at nodes, (4) Cd remobilization from leaves, and finally, (5) transportation into the grain (Uraguchi and Fujiwara, 2012; H. Li et al., 2017). The transport of Cd from the apoplast into the root cells mainly involves Nramp5, Nramp1, IRT1/IRT2, ZIP4, LCT1, and YSL (Sasaki et al., 2012; Qin et al., 2020). Some measures, such as knockout of *OsNramp5*, are applied to attain low Cd-accumulating rice cultivars (L. Tang et al., 2017), but whether intercropping affects the expression of related Cd transporters in rice roots remains unclear.

Therefore, this study aimed to investigate the effects of an *S. nigrum* and upland rice intercropping system inoculated with and without AMF on plant growth, Cd accumulation, root morphological change, organic acid contents, pH change in the rhizosphere, and gene expression of the related Cd transporters in rice when spiked with Cd. We hypothesized that (i) AMF inoculation would improve Cd uptake via the improvement of *S. nigrum* growth, increase organic acid and root parameters (including the total root length, the different root diameter ranges, and the root surface area), and reduce the rhizosphere pH of *S. nigrum*, ultimately accelerating Cd removal in the intercropping system, that (ii) the intercropping system coupled with AMF would decrease the Cd concentration in various parts of upland rice by the downregulation of Cd transporter genes in the root, increase the rhizosphere pH, and reduce the rhizospheric organic acid fractions, the root length, and root surface area, and that (iii) a significant competitive depletion zone of available Cd could be observed in the upland rice/*S. nigrum* intercropping system with AMF. Our findings provided a theoretical basis for further understanding of the combined use of intercropping and AMF inoculation to improve phytoremediation in Cd-contaminated soils.

2. Materials and methods

2.1. Plant cultivation

The rice seeds used in this experiment were upland rice (Hanyou 73),

obtained from Shanghai Tiangu Biotechnology Co., LTD. Hanyou 73 is a three-line Indica hybrid with water-saving, drought-resistant, and wide adaptability traits. The seeds of *S. nigrum* were provided by the Institute of Applied Ecology, Chinese Academy of Sciences. These seeds were sterilized in 10% H₂O₂ for 15 min and then were rinsed with deionized water several times. Subsequently, the seeds were grown in sterile moistened sands with 50% Yoshida's solution (Yoshida, 1976). The macroelement compositions (M) of Yoshida's solution were N (1.1419), P (0.258), K (0.4097), Mg (1.1315), Ca (0.7982), and S (1.541); the microelement compositions (mM) were Fe (28.487), Mn (6.428), B (15.105), Cu (0.1242), Zn (0.1217), and Mo (0.0599); and the Si supplement was 15.834 M. After 30 days, uniform seedlings of upland rice and *S. nigrum* were selected for the pot trials.

2.2. Soil and AMF preparation

The soil was collected from the experimental field of the Ecology Department, South China Agricultural University (23°16'N, 113°37'E), Guangzhou, PR China. The collected soil sample was dried naturally and sifted through a 2 mm sieve, without any soil gravel, leaves, or other debris. The tested soil contained 19.60 g/kg organic matter content, 0.09 g/kg available N, 0.17 g/kg available P, and 0.12 g/kg available K, with a pH value of 7.02. The soil pH, the soil carbon content, and the available content of N, P, K were determined by the method of Bao (Bao, 2008). The total Cd content in the soil was 1.12 mg/kg and was supplied as CdCl₂·5/2H₂O. The tested soil was thoroughly blended with the CdCl₂·5/2H₂O for a month to stay homogeneous. And the air-dried soil was autoclaved at 121 °C for 120 min to eliminate indigenous AMF. The available Cd content (DTPA-Cd) in the tested soil was 0.21 mg/kg.

One type of arbuscular mycorrhizal fungi, *Funnelliformis mosseae*, provided by the College of Agriculture, Guangxi University, was used in this experiment. This AMF was propagated on the roots of maize plants in a pot culture for 12 weeks. The inoculum contained a growth medium of spores and infected maize root fragments (Wang et al., 2008).

2.3. Experimental setup

2.3.1. Pot experiment

The pot experiment started in June 2019. The uniform cultivated seedlings of upland rice (height: ~12 cm) and *S. nigrum* (~8 cm) were transferred into each pot (length × width × height = 30 × 16 × 13 cm) with 3.0 kg soil (soil: sand = 4:1). Inoculum (100 g) was mixed with the 1/3–2/3 depth soil for each inoculated seedling (Luo et al., 2017). The pot experiment was conducted in a completely randomized design with the following variables: upland rice monoculture (inoculation without or with AMF), *S. nigrum* monoculture (inoculation without or with AMF), and upland rice/*S. nigrum* intercropping culture (inoculation without or with AMF), with seven replicates in each treatment, for a total of 42 pots (Fig. S1). The soil was maintained at 70% holding water capacity during the growing period. Yoshida's nutrient solution (20%) was added to each pot every week to maintain an adequate soil nutrient level for plant growth (Li et al., 2016).

2.3.2. Rhizo-box experiment

The rhizo-box experiment also started in June 2019. A total of 3.5 kg of soil (soil: sand = 4:1) was placed inside a 24 × 30 × 2 cm plastic rhizo-box, with two valves at the bottom for water drainage. The treatments were consistent with the treatment of the above pot experiment with 100 g inoculum (2.3.1). Treatments were the same as the pot experiments, with four replicates in each treatment (Fig. S2). Growing management was consistent with the above pot experiment (2.3.1).

2.4. Sampling

Samples of the pot experiment were harvested in August (Heading stage of upland rice, 56 days) and October (Maturity stage of upland

rice, 118 days), 2019, respectively. The rhizosphere exudates of rice and *S. nigrum* were carefully collected by the method described by Wen et al. (2017), with a 50 mL 0.2 mM CaCl₂ solution. Two 10-mL aliquots of soil suspension were acquired; one was for the measurement of the rhizosphere pH, and the other was stored at -40 °C for further analysis (Wen et al., 2017). Moreover, one replicate of each treatment in the heading stage was selected for non-destructive measurements of pH change around roots using the method of bromocresol purple (pH=5.2–6.8) in agar (Shane et al., 2006). Subsequently, the root and shoot parts of rice and *S. nigrum* were separated and then were rinsed successively with tap water and distilled water to remove the attached impurities. The rice plants were separated into the seed (hulled grain), husk, straw, and root at the ripening stage.

After measuring the fresh weight, one part of the root samples (~1.0 g) was stored in 50% ethanol for mycorrhizal colonization assessment (Luo et al., 2017). In addition, ~0.1 g of rice root subsamples were frozen in liquid N₂ and stored at -80 °C for the following analyses of Cd transporter genes. The remaining part of the root system was then used for root scanning by a root scanner (Epson Expression 1600 pro, Model EU-35, Japan) and analysed by WinRHIZO Reg2009 to acquire the root parameters (Yang et al., 2019a). Finally, the remaining root samples and the other parts of the plants were oven-dried at 70 °C to attain a constant weight.

As for the rhizo-box experiment, the plants were harvested in September 2019 (90 days). One replicate of each treatment was selected to harvest the soil and root samples using the grid method (length × width = 2 cm × 2 cm), namely, the rhizo-box was divided into 2 cm × 2 cm grids for soil samples, in total 180 soil pieces for each rhizo-box. Subsequently, the roots in the soil pieces were picked out carefully with tweezers and washed with tap water for several times before the root scanning to acquire the root parameters. Then the air-dry soil samples were extracted with 25 mL DTPA (diethylenetriaminepentaacetic acid) extractant to detect the DTPA-Cd content with the graphite furnace atomic absorption spectrometry (ZEEnit700P, Jena, Germany) (Zeng et al., 2019). The root and shoot parts of the other three replicates were harvested. The root parts were washed with tap water and distilled water for several times to remove the attached soil particles. Then the harvested samples were oven-dried at 70 °C for about a week to obtain a constant weight.

2.5. Element analysis and AM fungal colonization

The dried samples were ground in a stainless-steel mill and subjected to microwave digestion (MARS6, CEM Corporation, USA). At the same time, the quality control (CDHK-GBW(E)100349, certified reference material for the chemical composition of rice flour) and the blank samples were generated. The total Cd content was detected by inductively coupled plasma-mass spectrometry (ICP-MS, NexION 2000, USA). The recovery of the standard ranged between ~90% and ~120%.

The percentage of root length colonized by AMF was estimated by the method described by Luo et al. (2017) with slight modifications (Luo et al., 2017). The root samples stored in 50% ethanol were cut into ~1 cm segments, then soaked in 10% (w/w) KOH at 90 °C for 1 h in a water bath. Next, the roots were acidified with 5% lactic acid for 5 min and then stained with 0.05% Trypan Blue (w/w) at 90 °C for 30 min (Li et al., 2011). The AMF colonization rates were calculated using the grid-line intersect method (Giovannetti and Mosse, 1980). There were three replicates for each subsample.

2.6. Determination of organic acids in rhizospheric soil

The organic acid extracts were filtered with a PR cartridge and a 0.22 μm membrane to remove interfering substances in the extract. Then, the organic acid contents were measured by ion chromatography (ICS-900, DIONEX, USA) using an IonPac ICE-AS6 analytical column. The eluent was 0.4 mM heptafluorobutyric acid, with a flow rate of 1.0

mL/min. The regenerant was 5 mM tetrabutylammonium acid, and the sample loop volume was 50 μL (Baziramakenga et al., 1995). The recovery of the standard substances for the organic acids was 78.1% ± 0.17 (n = 3).

2.7. Expression analysis of Cd transporters in rice

Total RNA was extracted from the rice roots using the RNeasy plant Mini Kit (Qiagen, Hilden, Germany). The yield of RNA was determined using a NanoDrop 2000 spectrophotometer (Thermo Scientific, USA), and the integrity was evaluated by agarose gel electrophoresis stained with ethidium bromide. The RNA was reverse transcribed using the HiScript II Q RT SuperMix for qPCR (+gDNA wiper) (Vazyme, R223-01). *Nramp5*, *HMA3*, *HMA2*, and *GAPDH* (Glyceraldehyde 3-phosphate dehydrogenase, as an internal control) were amplified by a GeneAmp® PCR System 9700 (Applied Biosystems, USA) for real-time polymerase chain reaction (PCR). The designed primer pairs used in this study were listed in Table S1 (Chen et al., 2019). Relative expression of *Nramp5*, *HMA3*, and *HMA2* were calculated using the 2^{-ΔΔCt} method (Livak and Schmittgen, 2001).

2.8. Data analysis

Experimental data analysis was performed using a one-way, or two-way analysis of variance (ANOVA), and the means were compared using a significant difference (Duncan) method at a 5% level (SPSS 17.0). Before analyzing the parametric statistics (one-way or two-way ANOVA), the Shapiro-Wilk test and the Kolmogorov-Smirnov test (SPSS 17.0) were used to check the data normality. And SPSS 17.0 was used to perform the function conversion (Sqrt, LN, or Lg10 function) based on the results of the Shapiro-Wilk and Kolmogorov-Smirnov test (SPSS17.0). All data are showed as the mean ± standard error (n = 3). In addition, figures were generated using Sigmaplot for Windows Version 10.0.

The calculation of the bioconcentration factor (BCF) was as follows: $BCF = C_p / C_s$, where C_p and C_s refer to the Cd contents in the plant and soil, respectively, in mg/kg.

The calculation of transfer factors (TF) was as follows:

$TF = C_{\text{tissue1}} / C_{\text{tissue2}}$, where C_{tissue1} and C_{tissue2} refer to the Cd contents in the different parts of the rice plant, in mg/kg (Zeng et al., 2019).

The calculation of total Cd accumulation per plant was as followed: Total Cd accumulation per plant = $C_n \times B_n$, where n is a part of the plant (shoot, root), and B is the biomass of the relevant part of a plant.

The calculation of Seed Accumulation Factor (SAF) was as follows:

$SAF = C_{\text{seed or fruit}} / C_{\text{shoot}}$, where $C_{\text{seed or fruit}}$ and C_{shoot} refer to the Cd contents in the different parts of the rice plant, in mg/kg (Zhan et al., 2019).

(SAF < 1.0 indicates the prevalence of a protective mechanism against Cd accumulation in seeds. SAF < 0.5 indicates a high seed protection level. SAF = 1.0 shows that there is no specific protection against Cd accumulation in seeds. SAF > 1.0 indicates that seeds are particularly susceptible to Cd accumulation).

3. Results

3.1. Plant biomass

Both the cropping system and AMF treatments affected the biomass of upland rice and *S. nigrum* (Table 1). At the heading stage, the rice root and shoot biomass decreased significantly in the intercropping system with AMF addition. Compared with M treatment, the root and shoot biomass of IN + A was reduced by 80.0% and 84.7%, respectively. However, the rice root and shoot biomass showed no significant differences among M, IN, and M+A treatments at the heading stage. This showed that both the cropping system and AMF treatment significantly influenced the shoot biomass ($P < 0.05$). Similarly, at the maturity stage,

Table 1
Biomass of upland rice and *S. nigrum* under different treatments (g/plant DW).

Treatments	Upland rice			<i>S. nigrum</i>		
	Root	Shoot	Grain	Root	Shoot	Fruits
Heading stage						
M	0.2 ± 0.06a	3.66 ± 0.86a	–	0.19 ± 0.02b	4.68 ± 0.91b	0.41 ± 0.08ab
IN	0.22 ± 0.00a	2.98 ± 0.25a	–	0.49 ± 0.06a	5.52 ± 0.78b	0.32 ± 0.16b
M + A	0.23 ± 0.07a	2.71 ± 0.57a	–	0.45 ± 0.03a	5.91 ± 0.25ab	0.23 ± 0.07b
IN + A	0.04 ± 0.01b	0.56 ± 0.08b	–	0.57 ± 0.01a	8.35 ± 1.15a	0.73 ± 0.14a
Cropping	n.s.	**		*	n.s.	n.s.
AMF	n.s.	**		*	**	n.s.
Cropping × AMF	n.s.	n.s.		**	n.s.	**
Maturity stage						
M	0.30 ± 0.03a	4.50 ± 0.63a	4.85 ± 0.37a	0.12 ± 0.01b	5.94 ± 0.44b	0.37 ± 0.09b
IN	0.22 ± 0.05b	4.10 ± 0.32a	3.99 ± 0.71a	0.16 ± 0.03b	4.75 ± 0.53b	2.15 ± 0.07a
M + A	0.33 ± 0.05a	3.42 ± 0.13a	3.78 ± 0.48a	0.17 ± 0.02b	5.41 ± 0.66b	0.41 ± 0.03b
IN + A	0.12 ± 0.01b	1.49 ± 0.17b	1.00 ± 0.09b	0.41 ± 0.10a	10.39 ± 1.39a	0.69 ± 0.20b
Cropping	*	**	*	**	n.s.	*
AMF	n.s.	*	*	**	**	*
Cropping × AMF	n.s.	n.s.	n.s.	n.s.	*	*

M – monoculture, IN – upland rice/*S. nigrum* intercropping; +A – with AMF inoculation. Data of each row marked by the same lowercase letters for the same treatment are not significantly different at $P < 0.05$ (means ± SE, $n = 3$).

* denotes $P < 0.01$,

** denotes $P < 0.05$. n.s. denotes no significant difference.

the root, shoot, and grain biomass of IN + A were much lower than with the other three treatments. The root biomass under IN treatment decreased by 26.67%, compared with M treatment. However, the shoot and grain biomass showed no marked difference among M, IN, and M+A treatments. The two-way ANOVA indicated that both the cropping system and AMF treatment significantly affected the root (except AMF), shoot, and grain biomass of upland rice at the maturity stage, but the interaction of the cropping system and AMF showed no remarkable effect.

Inversely, markedly higher root, shoot, and fruit biomasses were observed in *S. nigrum* in the intercropping system with AMF addition (Table 1). The root, shoot, and fruit biomass of IN + A were 3.0-, 1.78-, and 1.78-fold that of M treatment, respectively, at the heading stage. Moreover, the root and shoot biomass of *S. nigrum* under IN + A were also the highest at the maturity stage, which was 3.42 and 1.75 times that of M treatment. The two-way ANOVA denoted that AMF treatment markedly increased the root and shoot biomass of *S. nigrum*. Similar results for the biomass of rice and *S. nigrum* were also observed in the rhizo-box experiment (Fig. S3).

3.2. Cd concentration and total Cd accumulation

The effects of intercropping and AMF treatments on the Cd concentration and total Cd accumulation in the shoot and root of upland rice were showed in Fig. 1a, b, c. Compared with monoculture treatments, the Cd concentration in rice shoots and roots markedly decreased by 80–87% and 54–81%, respectively, for the other treatments at the heading stage. The lowest Cd concentration in shoot and root was observed in the IN + A (Fig. 1a). Analogously, the Cd concentration in shoot, root, husk, and brown rice of upland rice were all remarkably reduced under the IN, M + A, and IN + A treatments, compared with M treatment (Fig. 1b). The lowest Cd concentration of all parts in upland

rice was observed in the IN + A group. Cd concentrations in brown rice were 1.073, 0.62, 0.40, and 0.38 mg/kg, respectively, for the M, IN, M + A, and IN + A treatments. Additionally, the lowest total Cd accumulation per plant was also observed in the IN + A group (Fig. 1c). Compared with the other three treatments, IN + A significantly decreased the total Cd accumulation in rice by 85.7–97.3%, 60.9–84.1% for the shoot, and 89.4–96.2%, 75.8–80.4% for root, respectively, at the heading and maturity stages.

In general, the superposition treatments showed no notable effects on the Cd concentration in the root, shoot, or fruit of *S. nigrum* (only IN notably enhanced the Cd concentration in the shoot and root) (Fig. 1d). Conversely, the highest total Cd accumulation in the root and shoot (including fruit) was all observed in the IN + A group, compared with the other three treatment groups at the heading stage (Fig. 1e). Comparably to M treatment, the total Cd accumulation in the root and shoot of IN + A increased by 179.2% and 84.4%, respectively, at the heading stage. At the maturity stage, only the shoot Cd accumulation of IN + A was significantly increased by 1.3-, 1.2-, and 1.9-fold that of the M, IN, and M + A treatments, respectively. Overall, the Cd concentration trends of upland rice and *S. nigrum* in the rhizo-box experiment were similar to those of potted upland rice and *S. nigrum*, respectively (Fig. S4).

3.3. Bioconcentration factors (BCF), transfer factors (TF), and seed accumulation factor (SAF) of plant

Both the cropping system and AMF treatments affected the BCF, TF, and SAF of *S. nigrum* and upland rice in different ways (Table 2). The highest $BCF_{shoot/soil}$ of *S. nigrum* was present in IN treatment, and the $BCF_{shoot/soil}$ of IN + A decreased significantly compared with M and IN treatments. The two-way ANOVA showed that the cropping system, the AMF, and the interaction of the cropping system and AMF all notably affected the $BCF_{shoot/soil}$ of *S. nigrum* at the maturity stage ($P < 0.05$). The $TF_{shoot/root}$ of *S. nigrum* in all treatments was higher than 1.0, but there was no obvious difference among different treatments. Besides, the $SAF_{fruit/shoot}$ of *S. nigrum* was all lower than 1.0, indicating a protective mechanism against Cd accumulation in seeds of *S. nigrum*.

For upland rice, the $BCF_{shoot/soil}$ of M treatment was markedly higher than that of IN, M + A, and IN + A treatments at the heading stage. In addition, the lowest $BCF_{shoot/soil}$ was exhibited in M + A and IN + A treatments, reducing by 67.5% and 45.2%, respectively compared with M and IN treatment. The two-way ANOVA indicated that the cropping system, the AMF, and the interaction of the cropping system and AMF all significantly affected the $BCF_{shoot/soil}$ of rice ($P < 0.01$). Additionally, only the $TF_{shoot/root}$ of M + A was markedly lower than M treatment at the heading stage, but the $TF_{shoot/root}$ of IN, M + A, and IN + A treatments were all notably lower than M treatment at the maturity stage. Based on the two-way ANOVA, the cropping system, the AMF, and the interaction of the cropping system and the AMF, all remarkably influenced the $TF_{shoot/root}$ of rice at the maturity stage ($P < 0.01$). Noticeably, the $SAF_{seed/shoot}$ of rice was lower than 0.5, indicating a high seed protection level in upland rice.

3.4. Total root length and root length in different root diameter ranges

The cropping system and AMF treatments differentially affected the total root length and the root length in different root diameter ranges of upland rice and *S. nigrum* (Fig. 2). In general, the total root length of *S. nigrum* showed a tendency of IN + A > M + A > IN > M. The number of roots in the 0–0.2 mm root diameter range was the largest, accounting for approximately 47.9–57.0% and 61.0–75.9%, respectively, for the heading and maturity stages. Noticeably, comparably to M treatment, the root length in each root diameter range of *S. nigrum* was significantly higher with IN + A treatment (Fig. 2a, b).

Similarly, the largest root length in the 0–0.2 mm root diameter range was also observed in rice, accounting for 62.8–87.2% and

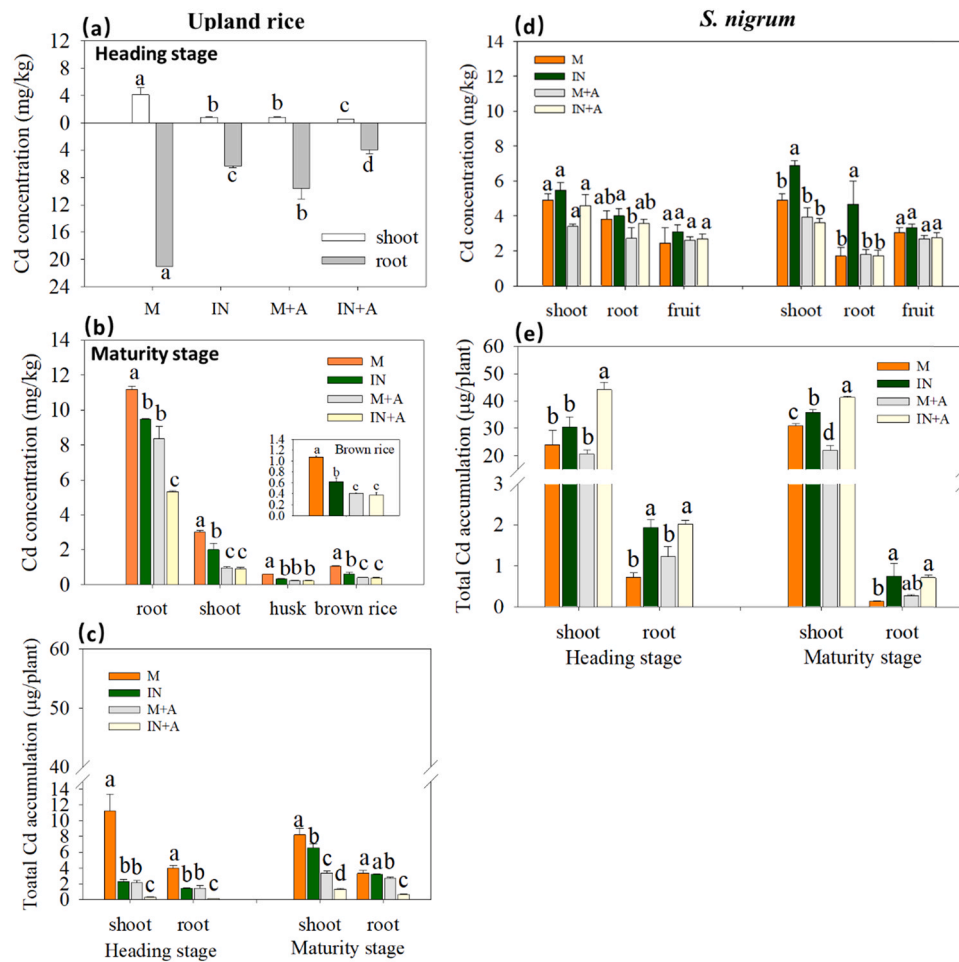


Fig. 1. Cd concentrations and total Cd accumulation in upland rice (a, b, c) and in *S. nigrum* (d, e) under different treatments. M – monoculture, IN - upland rice/*S. nigrum* intercropping; +A – with AMF inoculation. Data over bars marked by the same lowercase letters for the same treatment are not significantly different at $P < 0.05$ (means \pm SE, $n = 3$). * denotes $P < 0.05$.

Table 2

The BCF, TF, and SAF of *S. nigrum* and upland rice under different treatments.

Treatments	<i>S. nigrum</i>			Upland rice		
	BCF _{shoot/soil}	TF _{shoot/root}	SAF _{fruit/shoot}	BCF _{shoot/soil}	TF _{shoot/root}	SAF _{seed/shoot}
Heading stage						
M	4.38 \pm 0.33ab	1.35 \pm 0.25a	0.32 \pm 0.02c	2.94 \pm 0.43a	0.20 \pm 0.05a	
IN	5.53 \pm 0.75a	1.54 \pm 0.06a	0.50 \pm 0.02b	0.69 \pm 0.05b	0.12 \pm 0.01ab	
M + A	3.04 \pm 0.12b	1.37 \pm 0.26a	0.77 \pm 0.07a	0.73 \pm 0.08b	0.09 \pm 0.02b	
IN + A	4.09 \pm 0.59ab	1.27 \pm 0.09a	0.59 \pm 0.04b	0.47 \pm 0.01b	0.12 \pm 0.00ab	
Cropping	n.s.	n.s.	n.s.	*	n.s.	
AMF	**	n.s.	*	*	n.s.	
Cropping \times AMF	n.s.	n.s.	*	*	n.s.	
Maturity stage						
M	4.38 \pm 0.33b	3.19 \pm 0.55a	0.56 \pm 0.01c	2.68 \pm 0.08a	0.27 \pm 0.01a	0.36 \pm 0.02a
IN	6.16 \pm 0.24a	1.16 \pm 0.60b	0.48 \pm 0.01c	1.46 \pm 0.01b	0.17 \pm 0.00b	0.40 \pm 0.04a
M + A	3.52 \pm 0.45 BCE	2.24 \pm 0.27a	0.69 \pm 0.05b	0.87 \pm 0.04c	0.12 \pm 0.01c	0.42 \pm 0.03a
IN + A	3.23 \pm 0.21c	2.60 \pm 0.11a	0.90 \pm 0.05a	0.80 \pm 0.09c	0.15 \pm 0.00b	0.37 \pm 0.04a
Cropping	**	**	n.s.	*	*	n.s.
AMF	*	n.s.	*	*	*	n.s.
Cropping \times AMF	**	*	*	*	*	n.s.

M – monoculture, IN – upland rice/*S. nigrum* intercropping; +A – with AMF inoculation. Data of each row marked by the same lowercase letters for the same treatment are not significantly different at $P < 0.05$ (means \pm SE, $n = 3$).

* denotes $P < 0.01$,

** denotes $P < 0.05$. n.s. denotes no significant difference.

56.6–64.2%, respectively for the heading and maturity stages (Fig. 2c, d). For upland rice, under the intercropping conditions, the total root length was markedly reduced at the heading stage (Fig. 2c). The lowest

total root length and the root length in different root diameter ranges were also observed in IN + A. However, at the maturity stage, the differences in total root length between treatments were not significant

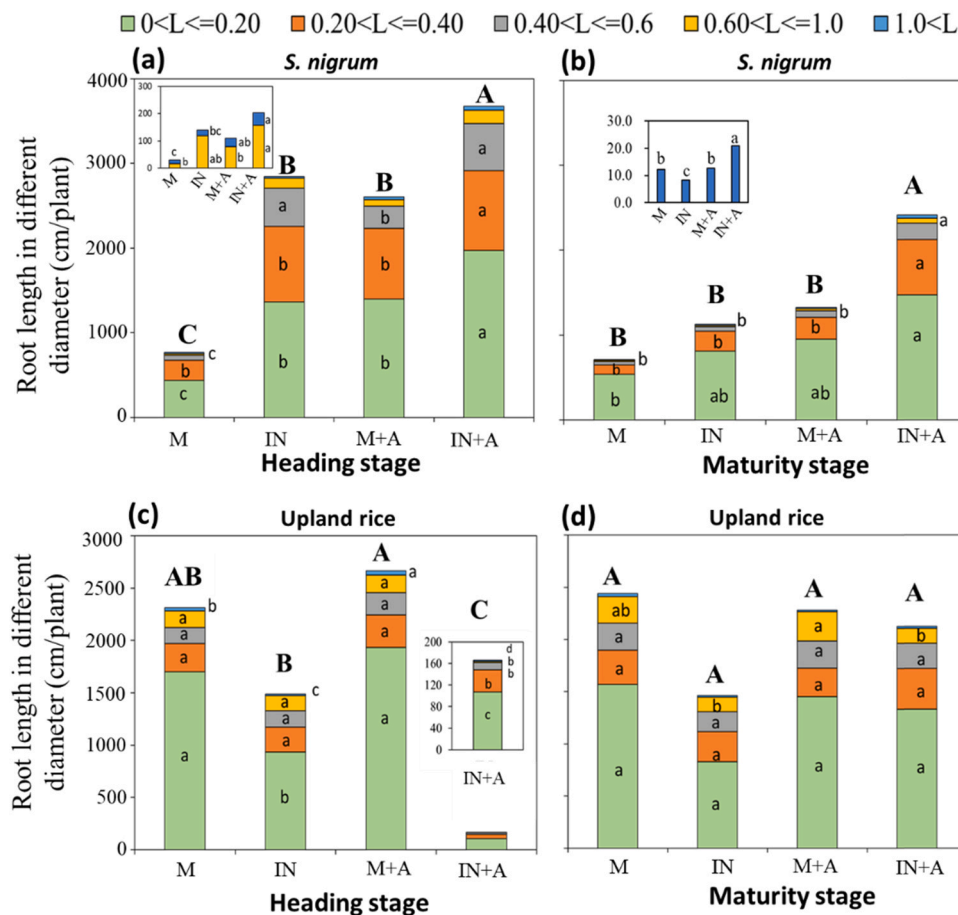


Fig. 2. Total root length in different diameters of *S. nigrum* (a, b) and upland rice (c, d) under different treatments. M – monoculture, IN – upland rice/*S. nigrum* intercropping; +A – with AMF inoculation. L – root length in different root diameter. Data over/under bars marked by the same lowercase/capital letters for the same treatment are not significantly different at $P < 0.05$ (means \pm SE, $n = 3$).

(Fig. 2d).

3.5. Spatial distribution of root surface area of upland rice and *S. nigrum*

The spatial distribution of roots in the rhizo-box was showed in Fig. 3. This indicated that the root surface area of *S. nigrum* and upland rice was markedly enhanced with the addition of AMF (Fig. 3a, c), compared to those without AMF (Fig. 3b, d). In the intercropping system, the root surface area of *S. nigrum* with/without AMF inoculation (Fig. 3e, f) was larger than that of sole *S. nigrum* without AMF (Fig. 3b). However, the rice root was significantly inhibited in the intercropping system (Fig. 3e, f), compared with sole rice with/without AMF (Fig. 3c, d). These results were consistent with the root length patterns in Fig. 2. This denoted an avoidant tendency in rice intercropped with *S. nigrum*, as more roots distributed in the right side of the rhizo-box (Fig. 3e, f), compared with sole rice cultivation.

3.6. Spatial distribution of DTPA-Cd in soils

The spatial distribution of DTPA-Cd of *S. nigrum* and rice in a rhizo-box under different treatments showed various patterns (Fig. 4). Compared to *S. nigrum* without AMF, the DTPA-Cd level in *S. nigrum* with AMF inoculation was higher, and this showed that the location with the highest Cd concentration in soil was coincidentally where the roots were the densest (Fig. 4a, b). Lower DTPA-Cd concentrations were observed in rice with AMF inoculation, showing a hollow of Cd in the rhizosphere, compared with rice without AMF (Fig. 4c). Noticeably, a clear Cd depletion zone appeared along with the root system in the

intercropping system with AMF inoculation (Fig. 4e), while a relatively higher DTPA-Cd content was observed in the intercropping system without AMF (Fig. 4f).

3.7. Changes in rhizosphere pH and rhizospheric organic acid fractions

The agar (initial pH of 5.6) was acidified by the root segments of *S. nigrum*. However, alkalization of the agar was observed in rice root segments (Fig. 5b, c). Moreover, the rhizosphere pH value of rice and *S. nigrum* showed similar patterns with the agar results. For upland rice, the lowest rhizosphere pH was observed in sole rice, while the pH of the intercropping system was markedly higher. However, for *S. nigrum*, the lowest rhizosphere pH was observed in the intercropping system with AMF inoculation. Overall, the rhizosphere pH of *S. nigrum* was significantly lower than that of rice (Fig. 5d).

The cropping and AMF treatments differentially affected the organic acid fractions in rhizospheric soil of rice and *S. nigrum* (Fig. 5e, f). For *S. nigrum*, the organic acid fractions in the rhizospheres were mainly oxalic, citric, and malic acids (Fig. 5e). Compared to M treatment, the citric and malic acids of IN + A were significantly higher. No marked difference in the oxalic acid content was observed between treatments. For upland rice, a significant increase of oxalic, citric, and malic acids was observed in rhizospheric soil in the M treatment, while the organic acid fractions of IN + A were below the detection level (Fig. 5f).

3.8. Relative expression of *Nramp5*, *HMA3*, and *HMA2* in rice root

The relative expression levels of *Nramp5*, *HMA3*, and *HMA2* in

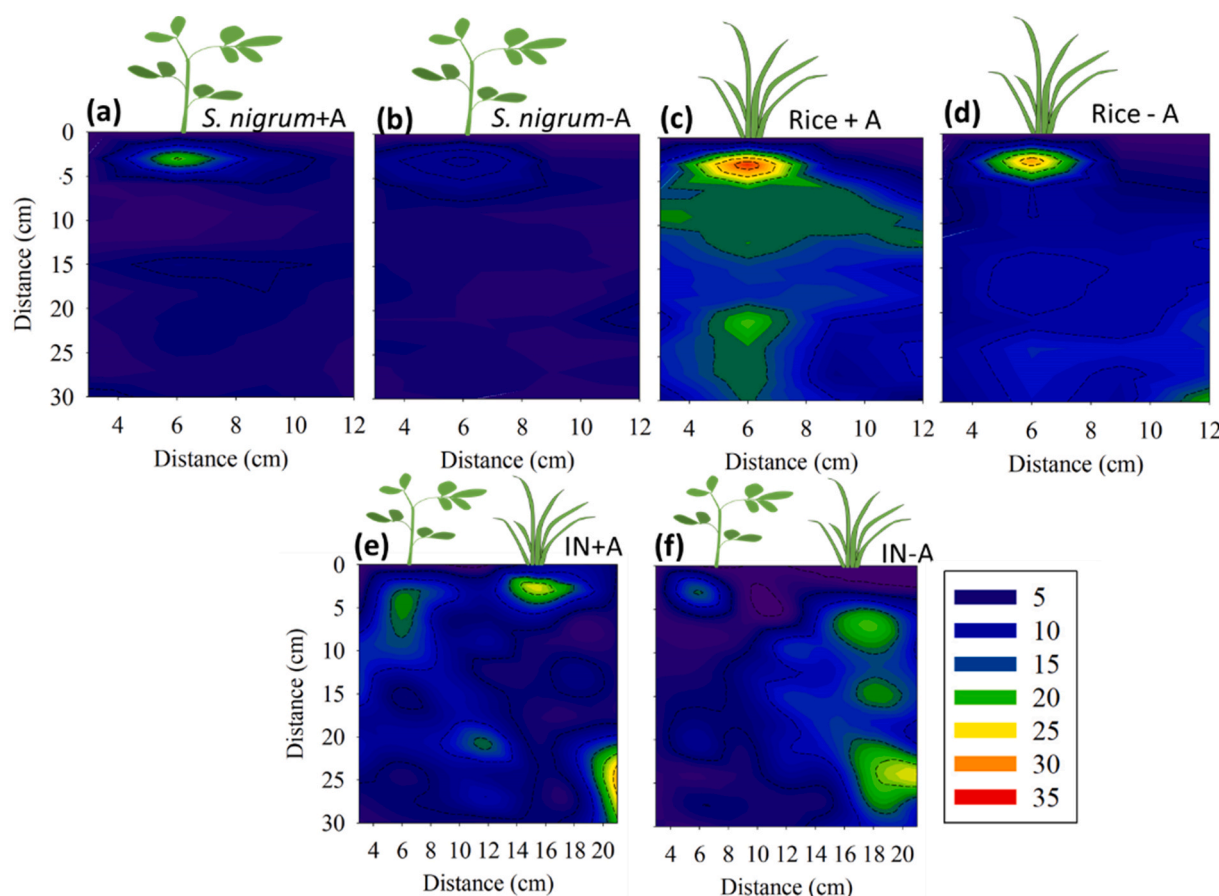


Fig. 3. Spatial distribution of root surface area of upland rice and *S. nigrum* under different treatments (cm²/plant).

upland rice roots showed a different pattern among treatments (Fig. 5). Compared to M treatment, the relative expression of *Nramp5* in rice roots did not significantly change in IN treatment, but a notable decrease was showed in IN + A. However, a significantly higher level of *Nramp5* was observed in M+A, compared with M treatment (Fig. 5a). Additionally, the relative expression of *HMA3* in IN, M + A, and IN + A treatments was markedly higher, compared with M treatment (Fig. 5b). However, *HMA2* expression indicated no significant differences among treatments, and only *HMA2* expression in IN + A was notably lower than that of IN treatment (Fig. 5c).

3.9. AMF colonization rate

For inoculated treatments, the AM fungal colonization rates ranged from 18.77% to 31.76%, while for the treatments without AMF, the colonization rate ranged from 2.03% to 3.76% (Table 3). Additionally, a significantly higher colonization rate was observed in plants inoculated with AMF, compared with no AMF colorization. However, the colorization rate of upland rice and *S. nigrum* showed no remarkable differences among different treatments.

4. Discussion

4.1. Effects of intercropping and AMF on plant biomass and AM fungal colonization

Numerous studies have showed that intercropping can improve the use efficiency of mineral nutrients, light, water, and space resources, thus enhancing crop yield/quality (Agegnehu et al., 2006; Lu et al., 2017). The yield advantages are commonly generated by complementary effects, better resource use efficiency, or buffering effects against

diseases and weeds (Hauggaard-Nielsen et al., 2008; Raseduzzaman and Jensen, 2017; Li et al., 2020). However, it has also been suggested that intercropping systems inoculated with AMF could sometimes result in unpredictable outcomes because of the growth and nutrients, and heavy metal uptake of the two intercropped plants. In this study, intercropping negatively affected upland rice biomass and rice grain, especially with AMF inoculation (Table 1). It indicated that the rice yield of IN + A was markedly decreased by 79.4% and 74.9%, respectively, compared with that of M and IN treatment. However, without AMF colonization, there was no significant difference between the M treatment (4.85 g/plant) and IN treatment (3.99 g/plant) groups. Conversely, the highest root and shoot biomass of *S. nigrum* were all observed in the IN + A group. This trend suggested that *S. nigrum* had a dominant advantage when competing with rice, and the addition of mycorrhiza further strengthened the competitive advantage of *S. nigrum* (Table 1). Though the land equivalent ratios (LER) of the upland rice/*S. nigrum* intercropping system with and without AMF were 2.28 and 1.95 (>1.0), respectively, it still denoted a lower yield in upland rice in the IN + A group. Huang et al. (2019) suggested that even if the sum of relative yields (LER) is often greater than one, the intercrop yields would also be lower than those obtained in sole crop production (Huang et al., 2019). Similar results from the wheat/maize and the maize/watermelon intercropping system in the field trials were showed by Gou et al. (2016) and Huang et al. (2018). Additionally, many studies have demonstrated that AMF inoculation promotes the plant growth of *S. nigrum* (Marques et al., 2008; Li et al., 2018; G. Wang et al., 2020). Therefore, in this controlled pot experiment, the reduced biomass in upland rice may mainly relate to the inevitable competition for nutrients, water, and space between upland rice and *S. nigrum* (Hu et al., 2017; X. Li et al., 2017). Furthermore, the total root length in *S. nigrum* was much higher than that of upland rice in IN + A at the heading stage (Fig. 2), and the root surface area

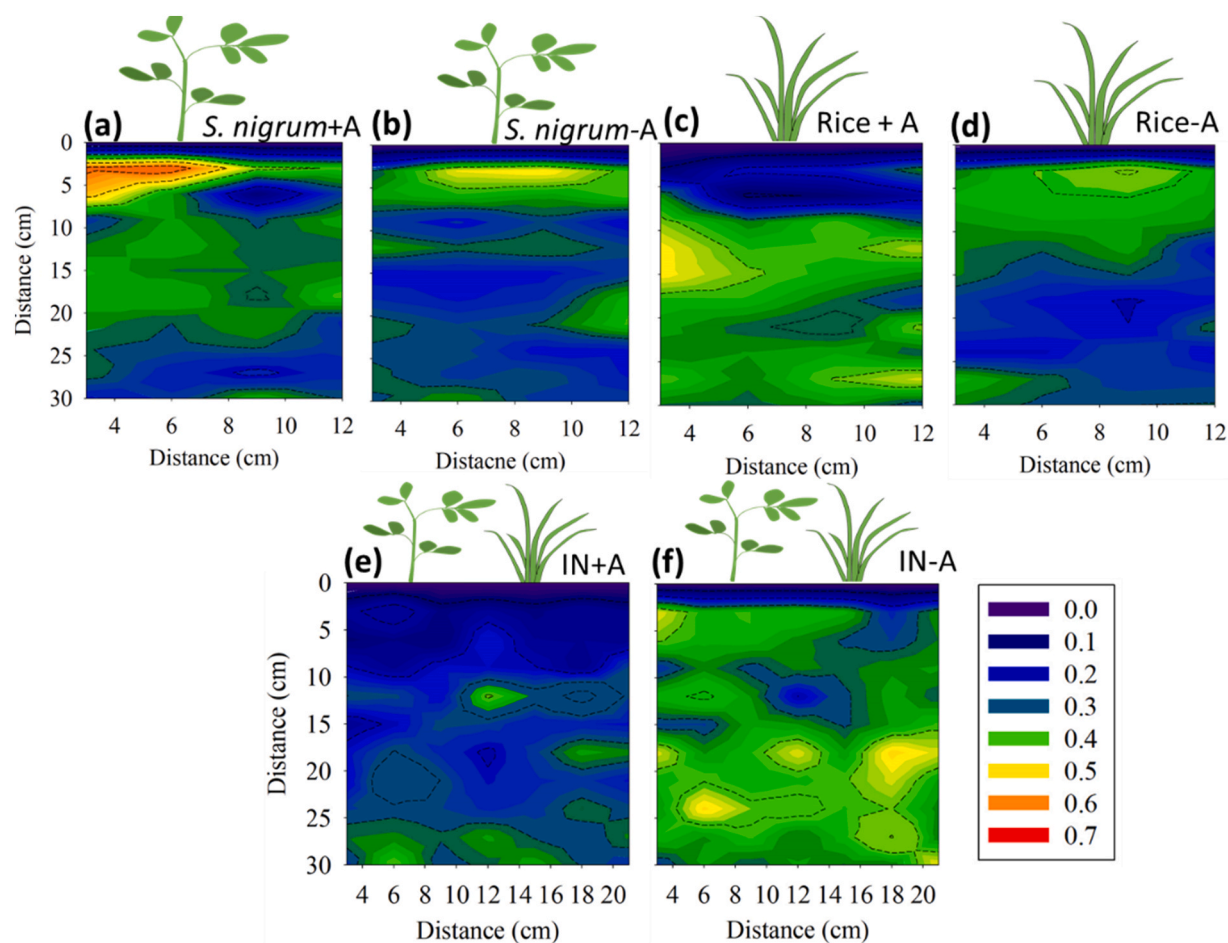


Fig. 4. Spatial distribution of DTPA-Cd in soils under different treatments (mg/kg).

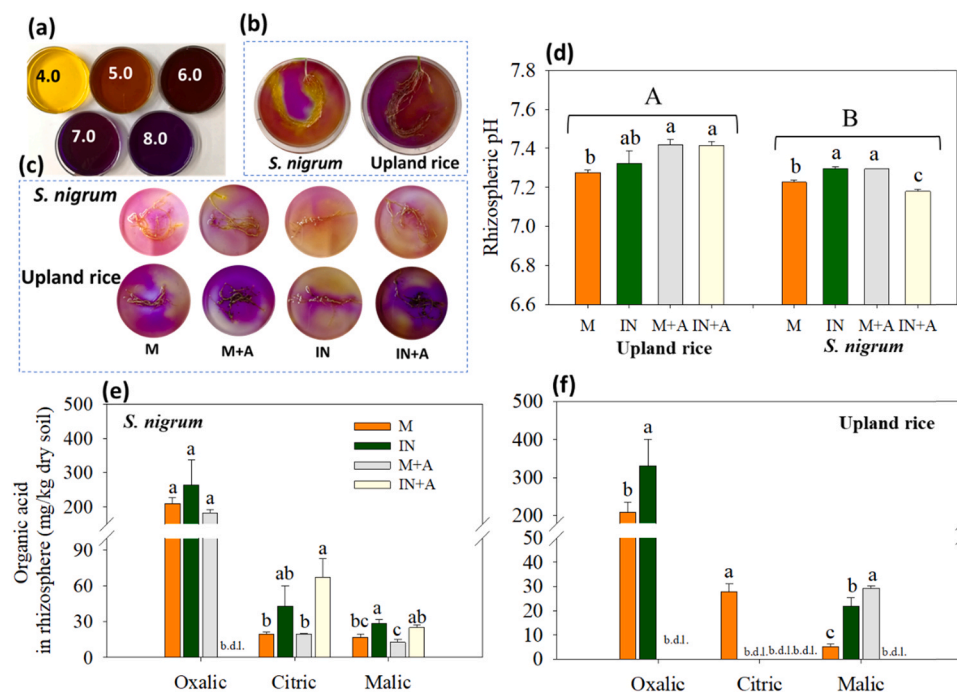


Fig. 5. Organic acid and pH value in the rhizosphere of upland rice and *S. nigrum*. (a) Agar standards with different pH levels; (b) unbroken *S. nigrum* and rice roots in agar with initial pH = 5.6; (c) *S. nigrum* and rice root segments under different treatments in agar with initial pH = 5.6; (d) rhizospheric pH value of *S. nigrum* and rice; (e) organic acid fractions in rhizospheric soil of *S. nigrum*; (f) organic acid fractions in rhizospheric soil of rice. b.d.l. denotes below detection levels. M – monoculture, IN – upland rice/*S. nigrum* intercropping; +A – with AMF inoculation. Data over bars marked by the same lowercase letters for the same treatment are not significantly different at $P < 0.05$ (means \pm SE, $n = 3$).

Table 3AM fungal colonization rate of upland rice and *S. nigrum* under different treatments.

Treatments	Upland rice		<i>S. nigrum</i>	
	Heading stage	Maturity stage	Heading stage	Maturity stage
M	3.10% ± 1.27b	2.19% ± 0.53b	3.23% ± 0.79b	3.39% ± 0.79b
IN	3.38% ± 0.23b	3.76% ± 0.42b	2.03% ± 0.48b	3.61% ± 1.32b
M + A	18.77% ± 6.41a	22.23% ± 3.88a	29.69% ± 4.38a	31.76% ± 5.42a
IN + A	24.60% ± 6.02a	19.20% ± 2.92a	27.68% ± 0.73a	23.82% ± 2.08a

M – monoculture, IN – upland rice/*S. nigrum* intercropping; +A – with AMF inoculation. Data of each row marked by the same lowercase letters for the same treatment are not significantly different at $P < 0.05$ (means ± SE, $n = 3$).

distribution also proved that the root growth of rice was inhibited in the intercropping system with AMF inoculation (Fig. 3). In addition, it denoted that total root length was positively correlated with the biomass of upland rice ($P < 0.01$) and of *S. nigrum* ($P < 0.01$) (Craine and Dybzinski, 2013).

Successful AMF colonization was observed in the plant roots (Table 3). The root colonization rates in the mycorrhizal treatments were significantly higher than those without mycorrhizal inoculation. However, the AM fungal colonization rates in this study were lower than some reported results, which ranged from 32%–43% (Chen et al., 2019) or 49.5–72.4% (G. Wang et al., 2020). We speculate that this may be related to a relatively high background value of soil phosphorus (P) content in this study, as mycorrhiza is more likely to form a symbiotic relationship with plants at a low P level (Kazantseva et al., 2009). It could also be related to differences in various plant species, the soil condition, etc. (Hu et al., 2013; Zhang et al., 2019).

4.2. Effects of intercropping and AMF on BCF, TF, SAF, root parameters, pH, and organic acid content

TF is an important indicator for assessing the phytoextraction abilities of pollutants by plants, and BCF is an essential index for the permitted limit of contaminants for different crops (Tang et al., 2020). In this study, the TF of *S. nigrum* in all treatments was higher than 1.0, identifying the hyperaccumulating features of *S. nigrum* (Kacálková et al., 2015). In contrast, the intercropping and AMF treatments both decreased the BCF and TF values of upland rice, suggesting a lower Cd uptake and translocation in rice. This was probably related to the changes in expression of some critical trace element transporters, such as HMA and Nramp families (Tiong et al., 2015), which is in line with our gene expression results for the Cd transporters (Fig. 6). These results are well supported by the Cd acquisition results in rice (Fig. 1). Additionally, the SAF_{seed/shoot} of upland rice was lower than 0.5, showing a protective mechanism against Cd accumulation in seeds of upland rice (Zhan et al., 2019).

It is suggested that coarse roots retain more Cd in the tissue, while longer roots or more fine roots promote Cd uptake in plants (Huang et al., 2015; Yang et al., 2019a). Our results showed that the total root length, the root surface area, and fine roots in the 0–0.2 mm range were significantly higher in *S. nigrum* in the intercropping system with AMF inoculation. These results were in agreement with the root surface area in Fig. 3. This is probably attributed to the improvement of mineral nutrients, photosynthesis, and stress resistance of *S. nigrum* with AMF, while upland rice was at a competitive disadvantage (He et al., 2019). The total root length and fine root length of *S. nigrum* were positively related to the shoot Cd acquisition ($P < 0.05$). Additionally, Li et al. (2009) found that the thinner root of the hyperaccumulating ecotype was mainly constituted by roots with diameters between 0.2 and 0.4 mm (Li et al., 2009). J. Wang et al. (2020) showed that the thinner roots in the alligator flag contribute to larger Cd accumulation in the rice/alligator flag intercropping system.

The secretion of root exudates is one of the important mechanisms for altering the rhizosphere to promote metal acquisition in plants, especially for the hyperaccumulator (Rajniak et al., 2018). For example,

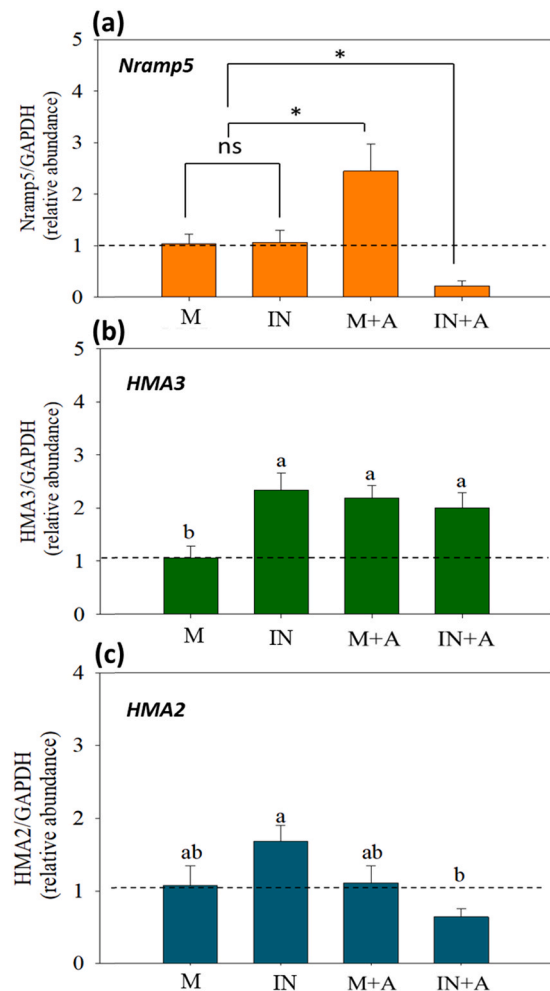


Fig. 6. The relative abundances of *Nramp5*, *HMA3*, and *HMA2* genes in upland rice roots under different treatments, compared to monoculture without AMF colonization (M treatment). M -monoculture, IN -upland rice/*S. nigrum* intercropping; +A – with AMF inoculation. Data over bars marked by the same lowercase letters for the same treatment are not significantly different at $P < 0.05$ (means ± SE, $n = 3$). ** denotes $P < 0.01$, * denotes $P < 0.05$. n.s. denotes no significant difference.

Tao et al. (2020) proved that tartaric acid enhances Cd mobilization and uptake in the Cd-hyperaccumulator *Sedum alfredii* (Tao et al., 2020). Bao et al. (2011) indicated that *S. nigrum* with higher organic acid concentrations in roots accumulated more Cd in the plants (Bao et al., 2011). This speculates that the increased organic acid contents lead to rhizosphere soil acidification, and decreased soil pH value (Fig. 5d), further improving the metal availability (Zu et al., 2020). It has been proven that a decrease in pH of only 0.2 units leads to a 3–5 times increase in the labile Cd pool (Zhu et al., 2016). In our results, the total organic acid content in *S. nigrum* was higher than that of upland rice, which was

consistent with the lower rhizosphere pH in *S. nigrum* (Fig. 5d). The greater organic acid content in *S. nigrum* may be related to the larger biomass with AMF inoculation. This subsequently leads to a higher production in photosynthate (Mathur et al., 2018), which partially transforms into a wide range of root exudates in the rhizosphere (Oldroyd, 2013). In addition, the inoculated AMF markedly decreased the release of organic acid content in rice, which probably led to lower Cd availability in the rhizosphere, thus reducing Cd uptake in rice tissues (Fig. 1). Furthermore, in this study, the main organic acid fractions of upland rice and *S. nigrum* were oxalic, citric, and malate acids, which probably related to the plant species and the different soil environments (Pearse et al., 2006).

4.3. Effects of intercropping and AMF on the expression of Cd transporters

Abundant documents have verified that Cd enters into root cells via the transporters NRAMP5 or IRT1, and NRAMP5 is predominantly applied in rice roots (Sasaki et al., 2012; Gao et al., 2016). Then, Cd is transported to the vacuole by OsHMA3 for compartmentalization (Miyadate et al., 2011), and the OsHMA2 transporter is involved in root-to-shoot translocation of Cd (Takahashi et al., 2012). In our results, the gene expression of *Nramp5* in the superposition treatment (IN + A) was significantly lower than that in monoculture (Fig. 5a), but the *Nramp5* expression in M + A was the highest. To our knowledge, this interesting result is found in the upland rice/*S. nigrum* intercropping system with AMF inoculation for the first time. Although a similar higher *Nramp5* level was observed in sole upland rice inoculated with *Funnelliformis mosseae*, compared with no-AMF treatment (Chen et al., 2019). However, the related researches on how the cropping system or AMF inoculation directly affects the gene expression of *Nramp5* is not clear yet. Based on our present work, we speculate that there are two possible mechanisms: (i) The intercropping and mycorrhizal symbiosis directly trigger and regulate the expression of related gene in rice root (Direct mechanism). For example, Zhou et al. (2020) suggested that *S. maltophilia* R5–5 inoculation may directly down-regulate the expression of *Nramp5* and *HMA2* in rice roots by its own antibiotic resistance phenotypes (Zhou et al., 2020). (ii) The expression of Cd transporters in rice roots is indirectly affected by feedback regulation of Mn concentration in rice root. *Nramp5* is a major transporter responsible for Mn and Cd uptake in rice roots (Sasaki et al., 2012). In our results, although the Cd concentration in the roots under the IN + A treatment was the lowest, the Mn concentration was significantly higher than that of M and IN treatments (Fig. S5). Therefore, we speculate that the Mn concentration in rice roots negatively regulated the expression of *Nramp5*. Our results revealed a new close linkage among the intercropping, the AMF inoculation, and Mn concentration on the Cd transporter gene expression in upland rice. Besides, the *HMA3* expression was lowest in the sole rice without AMF inoculation, and relatively lower *HMA2* expression was observed in IN + A. This tendency coincidentally followed the Cd concentration (Fig. 1) and Cd transfer coefficients (Table 2) in rice. As far as we know, the regulation of *Nramp5*, *HMA3*, and *HMA2* genes expression upon intercropping and mycorrhizal symbiosis in upland rice was reported for the first time.

4.4. Effects of intercropping and AMF on Cd acquisition in upland rice and *S. nigrum*, and the phytoremediation potential

Many studies have considered intercropping as a promising alternative to address the heavy metal pollution in agricultural ecosystems (Cao et al., 2020). Our results indicated a marked decrease in Cd concentration in various parts of rice in the intercropping system with and without AMF inoculation (Fig. 1a, b). Inversely, the total Cd acquisition in *S. nigrum* was significantly increased by the intercropping and AMF treatments (Fig. 1e). However, the intercropping and AMF treatments showed no significant effects on the Cd concentration in shoots or roots

of *S. nigrum* (Fig. 1d), which was probably due to a notable dilution effect ($P < 0.05$) on the Cd concentration with the increased biomass (Table 1) (Hu et al., 2013).

Similar results (decreased Cd concentration in field crops, while increased Cd acquisition in hyperaccumulators) were observed in the intercropping system of pak choi and Sedum or fava bean and Sedum with inoculated endophytes (Ma et al., 2020; Tang et al., 2020), the intercropped *Pteris vittata*/*Morus alba* (Wan et al., 2017), and the intercropped *S. nigrum*/eggplant (Y. Tang et al., 2017). These results mentioned above were attributed to the increased root exudates, the decreased rhizosphere pH value in hyperaccumulator plants, the competition for phytoavailable Cd, or the change in the microbial community structure in the rhizosphere (Wan et al., 2017; Cao et al., 2020; Tao et al., 2020; Zhou et al., 2020). In our results, we perceived that the increased Cd acquisition in *S. nigrum* and the reduced Cd concentration in upland rice were also probably related to the competitive advantages of *S. nigrum* in this intercropping system inoculated with AMF. As suggested in Fig. 4, significantly lower DTPA-Cd was observed in the intercropping system with AMF inoculation (Fig. 4), indicating a competitive depletion zone between *S. nigrum* and rice (Wan et al., 2017). Moreover, the significant decrease in BCF and TF in rice (Table 2), the increase in total root length and absorptive surface area in *S. nigrum* (Figs. 2, 3), the release of total organic acid content (Fig. 5), and the downregulated expression of Cd transporter genes (*Nramp5*) (Fig. 6) also played important roles in reducing the Cd content in various parts of rice.

In this upland rice/*S. nigrum* system, the lowest Cd concentration of brown rice was showed in IN + A (0.38 mg/kg), reduced by 64.5%, compared with sole upland rice (1.07 mg/kg) without AMF. This value was lower than the limit established by the Codex Alimentarius Commission of FAO/WHO (≤ 0.4 mg/kg, CXS 193–1995) and the Hygienical standard for feeds of China (≤ 0.5 mg/kg, GB 13078–2017). Although this upland rice/*S. nigrum* system with AMF inoculation did not guarantee rice production, it guaranteed food security and increased biomass of the neighbouring *S. nigrum*. Besides, the land-use efficiency of intercropping was higher than that of sole cropping of upland rice or *S. nigrum*, as the LER of the intercropping system with and without AMF were much greater than 1.0 (2.28 and 1.95, respectively). The total Cd accumulation in *S. nigrum* under IN + A treatment was 42.01 $\mu\text{g/plant}$, which was significantly higher than that of M, IN, M+A treatments (31.04, 36.51, 22.20 $\mu\text{g/plant}$ respectively). Taken together, the system of upland rice/*S. nigrum* with AMF inoculation indicated interesting results, it reduced the grain Cd concentration while increased the Cd phytoremediation, the land-use efficiency, and the biomass of *S. nigrum* in this work, but with a disadvantage of reducing the rice yield. However, there are unavoidable environment differences (including light, water, temperature) between the lab and field experiments, which account for unexpected results in plant performance between the field and the lab condition (Poorter et al., 2016; Xu et al., 2019). Therefore, the potted system may not be representative enough because Cd uptake could be affected by various conditions (such as soil property, crop category, and climate conditions, etc.) (Puschenreiter et al., 2005). Hence, more field trials are needed to verify the practicality of this upland rice-*S. nigrum* system with AMF inoculation.

5. Conclusions and prospects

This study illustrated that intercropping upland rice with the hyperaccumulator *S. nigrum*, coupled with AMF inoculation, resulted in the lowest Cd concentration in various parts of the rice plants, and the Cd concentration in brown rice met the FAO/WHO standard (≤ 0.4 mg/kg). This decrease was achieved because of the competitive Cd uptake of the neighbouring *S. nigrum*, with a significant competitive depletion zone (lower DTPA-Cd) in the intercropping system with AMF inoculation. Moreover, the superposition treatments markedly downregulated *Nramp5* gene expression, while upregulating the *HMA3* gene in rice

roots, resulting in lower Cd bioaccumulation and transfer coefficients. Besides, fewer root parameters (root length and root surface area) and lower organic acid exudation also led to lower Cd concentration in rice parts. In contrast, the coupled treatments of intercropping and AMF inoculation promoted biomass production, as well as total root length and fine root length in the 0–0.2 mm diameter range in *S. nigrum*, and lower rhizosphere pH with higher rhizospheric organic acids in *S. nigrum*, thus increasing total Cd accumulation. Our results also provided a better understanding of the role of AMF in the upland rice/*S. nigrum* intercropping system in Cd-polluted soils. Although the upland rice/*S. nigrum* with AMF inoculation showed a negative effect on the rice yield, it reduced the grain Cd concentration while increased the Cd phytoremediation, the land-use efficiency, and the biomass of *S. nigrum*. It still presents phytoremediation potential for the Cd-contaminated area.

In sum, the above results provided a better understanding of the possible regulatory mechanisms of the intercropping of hyperaccumulators and crops. However, more field experiments are needed to evaluate the feasibility of this upland rice-*S. nigrum* system with AMF inoculation. Further in-depth studies at the rhizosphere processes, the root-root interaction, and the plant-microbe interaction of the hyperaccumulator-cash crops intercropping system with mycorrhiza will provide insights into accelerating the remediation efficiency of phytoremediation. Moreover, further research into the soil types, the species of mycorrhiza, hyperaccumulators, and cash crops would help to fulfil the whole picture of phytoremediation coupled with intercropping and mycorrhiza. The information gained from such research would contribute to considerably improving the phytoremediation potential of the heavy metal contaminated soil.

CRedit authorship contribution statement

Xu Yang: Conceptualization, Data curation, Formal analysis, Software, Writing - original draft, Writing - review & editing. **Junhao Qin:** Conceptualization, Writing - review & editing. **Jiachun Li:** Methodology, Data curation, Investigation. **Zhenai Lai:** Methodology, Data curation, Investigation. **Huashou Li:** Conceptualization, Funding acquisition, Data curation, Project administration, Resources, Supervision, Writing - review & editing.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at [doi:10.1016/j.jhazmat.2020.124325](https://doi.org/10.1016/j.jhazmat.2020.124325).

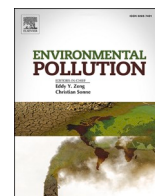
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Mycorrhizal fungi drive Cd and P allocation strategies for the co-planting system of hyperaccumulator *S. nigrum* and upland rice[☆]

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ABSTRACT

Arbuscular mycorrhizal fungi (AMF) enhance the remediation potential of hyperaccumulator-crop co-planting systems, yet the mechanisms governing cadmium (Cd) and phosphorus (P) allocation remain unclear. To investigate these strategies, pot experiments were conducted using Cd-contaminated soil (1.0 mg kg⁻¹ Cd) where the Cd hyperaccumulator *Solanum nigrum* (*S. nigrum*) was intercropped with upland rice under *Funneliformis mosseae* inoculation. Rhizospheric GRSP content, Cd/P allocation patterns, and microbial community structure were analyzed using in situ analysis using laser ablation inductively coupled plasma mass spectrometry (LA-ICP-MS), sequential chemical extraction, and 16S rRNA sequencing. Results showed that AMF increased total Cd accumulation in *S. nigrum* shoots by 25.37 % while reducing Cd uptake in rice shoots and roots by 45.18 % and 55.54 %, respectively. AMF also enhanced the P uptake rate of *S. nigrum* by 1.76 times compared to non-inoculated conditions, thereby increasing the total P accumulation in *S. nigrum* by 25.62 % under Cd stress. Conversely, AMF negatively impacted the P content and total P accumulation in neighboring rice. Rhizospheric GRSP content increased significantly, indicating AMF's role in reducing Cd availability for rice. In situ analysis of LA-ICP-MS confirmed lower Cd content in rice rhizosphere and root surfaces, with minimal effects on *S. nigrum*. Lower DTPA-Cd concentrations in the rhizosphere of intercropped rice further substantiated the mycorrhizal Cd-blocking effects of AMF. Furthermore, AMF inoculation was the principal factor influencing alterations in the bacterial community structure within the intercropping system, by increasing the abundance of phosphate-solubilizing bacteria (mainly *Ramlibacter*, *Roseisolibacter*, and *Bacillus*) in the rhizosphere. AMF reduced the relative abundance of metal-tolerant bacteria (primarily *Flavisolibacter*) in the *S. nigrum* rhizosphere while enhancing their presence in the rice rhizosphere. This work revealed the resource acquisition effect (especially P uptake) of AMF on *S. nigrum*, thereby promoting Cd uptake and its preferential strengthening of the Cd-defending effect of the intercropped rice.

1. Introduction

The co-planting of hyperaccumulating plants and crops typically increases the biomass and metal uptake of hyperaccumulating plants, while reducing the absorption of metals by target plants; This is a potential method for repairing contaminated soil without affecting agricultural production (Luo et al., 2019; Wan et al., 2023; Xia et al., 2018).

Co-planting combinations of hyperaccumulators and crops vary according to the purpose of safety cropping or soil cleanup. Increasing greenhouse and field studies suggest that co-planting hyperaccumulators and crops is a sustainable strategy for safely utilizing contaminated soil. Some commonly used combinations include arsenic (As) hyperaccumulators *Pteris vittata* and Cd hyperaccumulators *Solanum nigrum* (Yang et al., 2021), *Sedum alfredii* (Cao et al., 2021), etc., with

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crops such as *Zea mays*, *Vicia faba*, *Morus alba*, and *Oryza sativa* (Kang et al., 2020; Wan et al., 2017; Zu et al., 2017). Research has shown that root exudate, microbial communities, and root foraging behaviors often coexist and work together to directly or indirectly affect metal availability by shaping the rhizospheric properties in the co-planting system of hyperaccumulators and crops (Wan et al., 2023), including through changes in root exudate components (organic acids and amino acids with carboxyl functional groups), changes in soil pH and oxidation-reduction potential under intercropping mode (Wan et al., 2023; Yang et al., 2021; Zhan et al., 2016), effects on soil microorganism recruitment (Wang et al., 2020b) and the ability to absorb and transport heavy metals (Wan et al., 2017).

In other cases, with the low bioavailability of heavy metals in soil and the slow growth of hyperaccumulating plants, the phytoremediation efficiency of the co-planting system is still limited. Mycorrhizal associations are present in almost all ecosystems and more than 80% of terrestrial plant species form symbioses with arbuscular mycorrhizal fungi (AMF) (Smith and Smith, 2011). The addition of growth-promoting microorganisms (e.g., AMF) is widely used to improve extraction efficiency (Ma et al., 2020; Tang et al., 2020; Zhu et al., 2023). For example, *S. nigrum* inoculated with AMF ameliorates growth by enhancing productivity and nutrient acquisition and reducing the pH to increase Cd bioavailability in the soil, thus increasing plant Cd uptake (Wang et al., 2020a). Interestingly, the literature suggests that AMF inoculation further promotes heavy metal extraction by hyperaccumulating plants and reduces the heavy metal uptake of intercropped plants. For example, our previous work proved that AMF significantly enhanced the efficiency of extracting soil Cd from the Cd-hyperaccumulator *S. nigrum* and reduced the Cd concentration of the intercropped target rice (Yang et al., 2021). Wang et al., (2023a,b,c) also suggested that cocultivation with *S. nigrum* and inoculation with *Rhizophagus intraradices* could improve plant photosynthesis and antioxidant defense to alleviate Cd toxicity to the intercropped soybean (Wang et al., 2023c). The mentioned studies have mainly explained the effects of AMF inoculation on the intercropping system, but how AMF differentially affects the Cd uptake of the two neighboring plants still needs to be further explored.

Additionally, AMF has a good cooperative relationship with the host plant, with the hyphae having access to nutrient patches (especially P) and water outside the rhizosphere, beyond the influence of the root, and in soil pores that are inaccessible to roots (Jiang et al., 2021). AMF can contribute up to 80 % of the host plant's inorganic P requirements (Van Der Heijden and Horton, 2009; Wipf et al., 2019). In return, the host plant provides carbohydrates to ensure the growth of AMF (Millar and Bennett, 2016). The literature indicates that AMF also helps plants acquire more P content in the intercropping system. For example, AMF inoculation increases the P utilization in upland rice-mungbean intercropping systems (Xiao et al., 2010). Zhang et al. (2018) found that AMF promotes the P concentration of the intercropped alfalfa (Zhang et al., 2018). Lei et al. (2021) indicated that AMF enhanced plant growth and the P content of the intercropped upland rice and Cd-hyperaccumulator *S. calendulacea* (Lei et al., 2021). However, little is known about how AMF affects the allocation strategies of soil Cd and P in intercropped systems of hyperaccumulating plants and crops in Cd-contaminated soil.

AM fungi-bacterial interactions aid plant growth by improving nutrient access and enhancing tolerance to biotic and abiotic stresses (Pan et al., 2024; Wang et al., 2023b). AMF stimulates the growth of different microorganisms via hyphal secretions, by transferring the carbon of the photosynthetic products to the hyphae that are external to the soil (Emmett et al., 2021). Jin et al. (2024) indicated that AMF can prioritize recruiting mycorrhizal helper bacteria (MHB) from cultivable bacterial populations that can improve their P nutrition (Jin et al., 2024). Additionally, AMF also immobilizes heavy metals by changing rhizospheric microbial communities. For example, Chen et al. (2019) found that AMF lowers the soil Cd availability of rice by enhancing the relative abundance of Actinobacteria (Chen et al., 2019). Other similar

studies also indicate that AMF markedly affects key bacterial taxa (Actinobacteria and Fusobacteria) to improve *Medicago sativa* resistance to Cd stress (Wang et al., 2021). These works indicate that AMF works in conjunction with other soil bacteria to strengthen host plant growth and resistance to heavy metals. However, how AMF affects microbial recruitment to assist the acquisition of soil Cd and P in the intercropped hyperaccumulating plants and crops remains unclear.

Our previous study showed that intercropping *S. nigrum* with upland rice, when inoculated with AMF, is an effective system for Cd phytoremediation in farmland (Yang et al., 2021). However, the role of AMF in regulating the uptake of Cd and P in this system remains unclear. Therefore, the present study further investigated the AMF-mediated soil Cd and P uptake trade-off strategies in the intercropping system of Cd hyperaccumulator *S. nigrum* and upland rice (*Oryza sativa* L.). In-situ analyses of Cd and P availability and the microbial structure in the rhizosphere of this intercropping system were also performed. Using in situ analyses of Cd and P availability, rhizosphere microbial structure, and root surface dynamics, we aim to unravel the complex interactions governing resource allocation and Cd immobilization. The findings have practical implications for optimizing intercropping systems to simultaneously enhance phytoremediation efficiency and ensure crop safety and nutrient uptake in Cd-contaminated farmlands. Furthermore, this work contributes to theoretical advancements by elucidating the role of AMF in mediating plant-microbe-soil interactions, particularly in the context of resource competition and heavy metal defense. The following hypotheses were tested. 1) AMF inoculation induces distinct Cd and P allocation strategies between intercropped *S. nigrum* and rice by differentially regulating rhizosphere processes (e.g., GRSP secretion, microbial restructuring, and nutrient competition). This results in preferential Cd accumulation in *S. nigrum* and reduced Cd bioavailability for rice. 2) P nutrition of *S. nigrum* is governed by AMF addition, thereby enhancing Cd accumulation; that is, the addition of AMF has a resource acquisition effect (P uptake) for Cd-hyperaccumulator *S. nigrum* and strengthens the Cd-defending effect of the intercropped rice.

2. Materials and methods

2.1. Plant cultivation

The rice seeds used in this study were upland rice (*Oryza sativa* L., Hanyou 73, HY 73, Shanghai Tiangu Biotechnology Co., Ltd). *S. nigrum* seeds were provided by the Institute of Applied Ecology, Chinese Academy of Sciences. These seeds were sterilized in 10 % H₂O₂ for 15 min and were then rinsed with deionized water several times. Subsequently, the seeds were grown in sterile moistened sand, germinated seeds were watered with 50 % Hoagland nutrient solution (Cui et al., 2019). The nutrient solution was composed of (in μM) 2500 KNO₃, 2500 Ca(NO₃)₂·4H₂O, 1000 MgSO₄·7H₂O, 250 K₂SO₄, 80 Fe-Na-EDTA, 500 KH₂PO₄, 4.5 MnCl₂·4H₂O, 0.3 ZnSO₄·7H₂O, 0.16 CuSO₄·5H₂O, 0.16 (NH₄)₆Mo₇O₂₄·4H₂O, and 20 H₃BO₃. After 30 days, uniform seedlings of upland rice and *S. nigrum* were selected for the pot trials.

2.2. Soil and AMF preparation

Tested soil was collected from the experimental paddy field of South China Agricultural University (23°16'N, 113°37'E), Guangzhou, Guangdong Province, PR China. Each collected soil sample (paddy soil) was dried naturally and sifted through a 5 mm sieve to remove soil gravel, leaves, and other debris. The tested soil (soil: sand = 4:1) contained 18.80 g kg⁻¹ organic matter content, 44.10 mg kg⁻¹ available NO₃⁻, 6.65 mg kg⁻¹ available NH₄⁺, 0.06 g kg⁻¹ Olsen-P and 0.13 g kg⁻¹ available K and was pH 7.12. The soil pH, the soil carbon content, and the available N, P, and K content were determined via the method of Bao (Bao, 2008; Yang et al., 2019). The DTPA-Cd content in the soil was 0.11 mg kg⁻¹.

The experimental soil was thoroughly mixed with CdCl₂·5/2H₂O,

and two different Cd levels (0 and 1 mg kg⁻¹) were set for one month to make it uniform. The choice of this Cd concentration (1 mg kg⁻¹) was based on our previous research and the relatively high soil Cd concentrations in southern China (Hu et al., 2021; Yang et al., 2021). The air-dried soil was autoclaved at 121 °C for 120 min to eliminate native AMF. One type of AMF used in this study was *Funneliformis mosseae*, which was provided by the College of Agriculture, Guangxi University. This AMF was propagated in the roots of potted maize plants for 12 weeks. The inoculum contains the growth medium of spores and fragments of infected maize roots (Wang et al., 2008).

2.3. Experiment setup

The pot experiment was started in a greenhouse (16 h light/8 h dark, 25 °C/30 °C). Uniform seedlings of *S. nigrum* and upland rice (~8 cm) were transplanted into each pot (22 × 8 × 10 cm) with 2.0 kg of soil (soil: sand = 4:1) and 40 g of inoculum per plant. Based on our previous study, the intercropped *S. nigrum* with upland rice in the pot experiment followed a completely randomized design with two treatment variables: 1) Cd levels: 0 mg kg⁻¹ (control) and 1 mg kg⁻¹; 2) AMF inoculation: With or without *Funneliformis mosseae* inoculation. Four replicates were set for each treatment, with a total of 16 pots.

The rhizo-box experiment was conducted in a greenhouse (16 h light/8 h dark, 25 °C/30 °C). A total of 2.0 kg of soil (soil: sand = 4:1) was placed inside a 24 × 30 × 2 cm plastic rhizo-box, with two valves at the bottom for water drainage. The rhizo-box experiment was conducted in a completely randomized design with the following: 1) rice monoculture (inoculation without or with AMF), 2) *S. nigrum* monoculture (inoculation without or with AMF), and 3) upland rice/*S. nigrum* intercropped culture (inoculation without or with AMF). Only one Cd level (1 mg kg⁻¹ Cd) was set in this rhizo-box experiment. There were four replicates in each treatment and a total of 24 pots. The treatments were supplied with 40 g inoculum per plant. By conducting both experiments simultaneously, we were able to corroborate findings across different scales (whole-plant vs. rhizosphere) and validate the consistency of AMF effects on Cd and P dynamics in the intercropping system.

The AMF inoculum used in the pot experiment or the rhizo-box experiment was mixed with 1/3–2/3 of the depth of the tested soil for each inoculated seedling (Luo et al., 2017). Every non-mycorrhizal potted seedling received an equivalent amount of 5 mL of filtrate of unsterilized AMF inoculum, meanwhile, all potted seedlings were supplied with 5 mL of filtrate of unsterilized primitive soil to provide the same microflora except for the absence of AMF. The filtrate was obtained by suspending 100 g of unsterilized inoculum (or unsterilized primitive soil) in 900 mL of sterile water and filtering through five-layer quantitative filter paper (Whatman No.1). This allowed the passage of common soil microbes but efficiently retained the spores and hyphae of AMF (Wang et al., 2016). The experimental soil was maintained at 60–70 % holding water capacity during the growth period. Low-P Hoagland nutrient solution (50 μM P) was added to each pot every week to maintain adequate soil P levels for plant growth (Cui et al., 2019; Li et al., 2016). All pots were watered with the same amount of nutrient solution.

2.4. Sampling and analysis

2.4.1. Plant harvest and pot experiment Cd/P determination

Pot experiment samples were harvested 56 days after planting (Booting stage). Upland rice and *S. nigrum* were separated into shoot and root, and the roots were rinsed with tap and distilled water to remove the attached impurities. After the fresh weight was determined, some root samples (~1.0 g) were stored in 50 % ethanol for the evaluation of mycorrhizal colonization (Luo et al., 2017). The remaining root samples and the rest of the plant were dried in an oven at 70 °C to achieve a constant weight.

Dried plant samples were ground in a stainless steel mill and

microwave digested (MARS6, CEM Corporation, USA). Quality control (CDHK-GBW(E)100349 rice flour chemical composition reference material) and blank samples were also prepared. Total Cd and P contents were determined by ICP-OES (Avio 500, PerkinElmer, Singapore) and ICP-MS (NexION 350D, PerkinElmer, USA) (Yang et al., 2021).

2.4.2. Plant harvest and parameter determinations of the rhizo-box experiment

2.4.2.1. In situ analysis by laser ablation inductively coupled plasma mass spectrometry (LA-ICP-MS). As for the rhizo-box experiment, the plants were harvested 72 days after planting. One replicate of each treatment was selected to harvest the root-soil interface samples for trace element mapping (LA-ICP-MS). The selected root-soil interface samples of *S. nigrum* and rice were carefully located and dug out (about 2 cm in length and 2 cm in width). These samples were then glued to glass slides and fixed with an epoxy curing agent. After drying, the samples were polished with a file until the root-soil interface was exposed for Cd and P mapping. The trace element mapping was performed using an NWR 193 nm ArF Excimer laser-ablation system coupled to an iCAP RQ ICPMS at the Guangzhou Tuoyan Analytical Technology Co. Ltd., Guangzhou, China. The ICP-MS was tuned using NIST 610 and NIST 612 standard glass to yield low oxide production rates. Helium carrier gas at 0.7 L min⁻¹ was fed into the cup and the aerosol was subsequently mixed with 0.79 L min⁻¹ Ar make-up gas. The raw isotope data were reduced using the 'Baseline Subtract' and 'Trace Elements' data reduction scheme (DRS), the latter running within the IOLITE package. In IOLITE, user-defined time intervals are established for the baseline correction procedure to calculate session-wide baseline-corrected values for each isotope. The trace elements were calibrated using NIST 610 as the external standard (Paton et al., 2011).

2.4.2.2. DTPA-Cd content and GRSP content of soil. An additional three replicates of roots and shoots were harvested. The root and shoot parts of rice and *S. nigrum* were separated. The shaking method was then employed to collect the rhizosphere soil adhering to the root surfaces of both plants (Wen et al., 2017). Subsequently, the roots were rinsed sequentially with tap water and distilled water to remove any attached impurities. The cleaned roots were then scanned using a root scanner (Epson Expression 1600 Pro, Model EU-35, Japan) and analyzed with WinRHIZO Reg2009 software to obtain root parameters. 25 mL of diethylenetriaminepentaacetic acid (DTPA) was used to extract air-dried samples of rhizosphere soil and the content of DTPA-Cd was determined by graphite furnace atomic absorption spectrometry (ZEEnit700P, Jena, Germany) (Zeng et al., 2019).

Glomalin-related soil protein (GRSP) is a glycoprotein produced in the soil by AMF and possesses the ability to reduce the bioavailability and toxicity of heavy metals such as Cu, Cd, and Pb. The extraction and determination of glomalin-related soil proteins (GRSP) were carried out according to the method of Malekzadeh et al. (2016). The easily extractable GRSP (EE-GRSP) was extracted from 1.00 g of air-dried bulk soil or rhizosphere soil with sodium citrate solution (pH 7.0, 20 mM, 8 mL), and the resulting mixture was autoclaved for 30 min at 121 °C (0.1 MPa). For total GRSP (T-GRSP), 1.00 g samples of air-dried bulk soil or rhizosphere soil were extracted with sodium citrate solution (pH 8.0, 50 mM, 8 mL), and the resulting mixture was autoclaved for 60 min at 121 °C (0.1 MPa). The supernatant was separated by centrifugation at 5000×g (EE-GRSP) and 6000×g (T-GRSP) for 20 min. The T-GRSP extraction was performed four times until the supernatant was colourless, then the supernatant was mixed well and centrifuged at 10000×g for 3 min.

The T-GRSP or EE-GRSP supernatant was reacted with an acidic Coomassie Brilliant Blue G-250 solution. Bovine serum protein was used as the standard, and its absorption at 596 nm was measured using a spectrophotometer. Then, the content of GRSP was calculated according

to the standard curve. After obtaining the absorbance value, the sample concentration was calculated ($\text{mg}\cdot\text{g}^{-1}$ dry soil) according to the standard curve fitting equation: $y = 20.921x - 0.2957$ ($R^2 = 0.999$) (Jia et al., 2016).

2.4.2.3. *S. nigrum* gene amplification and PacBio sequencing. Total soil DNA from the rhizosphere soil samples of upland rice and *S. nigrum* in the intercropping system was extracted from fresh sample (3.0 g) using the PowerSoil® DNA Isolation kit (QIAGEN, Germany) according to the manufacturer's protocols. The near-full-length bacterial 16S rRNA gene (V1-V9) was amplified from samples using the primers 27F (GRGTTTGATYNTGGCTCAG) and 1492R (TASGGHT-ACCTTGTTASGACTT). The sequencing was performed on the PacBio sequencing platform and the SMRT sequence reads were processed through the SMRT Portal (SMRT Link, version 8.0) to filter sequences for length (<1340 or >1640 bp) and quality. By filtering the circular consumption sequencing (CCS) sequence using UCHIME, version 8.1, optimised CCS was obtained (using USEARCH, version 10.0) for operational taxonomic unit (OTU) clustering. The database for species annotation and taxonomic analysis was Silva (Release132, <http://www.arb-silva.de>; (Wang et al., 2007). To compare the bacterial communities in different samples, an analysis of similarity (ANOSIM) was performed

using PRIMER 6 (Zheng et al., 2020). The β -analysis with the principal component analysis (PCoA) was used to visualize the similarity or dissimilarity of data.

2.5. AM fungal colonization

The percentage of root length colonized by AMF was estimated according to the method described by Cui et al. (2019), with slight modifications. Fresh root samples were stored in 50 % ethanol, cut into approximately 1 cm segments, and immersed in 10 % (w/w) KOH in a 90 °C water bath (20~30 min for *S. nigrum* roots, ~40 min for rice root). Next, the roots were stained with 5 % ink-vinegar solution for 5 min (Cui et al., 2019). The AMF colonization rate was calculated using the grid-line intersect method (Giovannetti and Mosse, 1980).

2.6. Data analysis

Experimental data analysis was performed using one-way or two-way analysis of variance (ANOVA). The means were compared using a significant difference (Duncan) method at a 5 % level (SPSS 17.0). All data are shown as the mean \pm standard error ($n = 3$ or 4). Figures were generated using Sigmaplot for Windows Version 10.0.

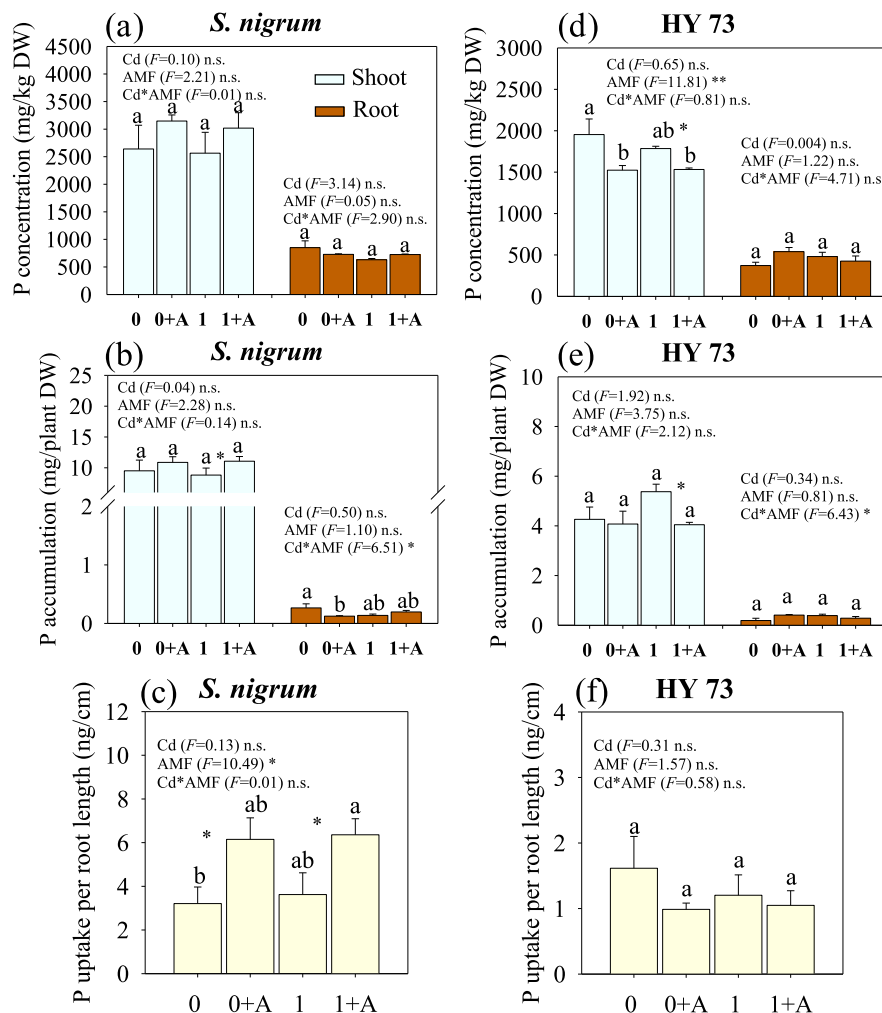


Fig. 1. P uptake patterns of interplanted *S. nigrum* and upland rice. (a) The P concentration of *S. nigrum* shoot and root; (b) The P accumulation of *S. nigrum* shoot and root; (c) The P uptake rate of *S. nigrum* shoot and root; (d) The P concentration of upland rice shoot and root; (e) The P accumulation of upland rice shoot and root; (f) The P uptake rate of upland rice shoot and root. +A-with AMF inoculation. 0- without Cd addition (control), 1- Cd level with 1 mg/kg. Each value is the mean (\pm SE) of three replicates. Different lowercase letters show statistically significant differences among different treatments ($P < 0.05$), and the t -test was run to assess the difference between AMF inoculation and no-AMF inoculation. * $P < 0.05$.

3. Results

3.1. Plant P accumulation and P uptake rate

AMF inoculation resulted in a significantly higher colonization rate compared to non-inoculated plants, as depicted in Fig. S1. However, the impact of AMF on the biomass of intercropped rice and *S. nigrum* was found to be minimal (Fig. S2), thereby eliminating the potential biological dilution effect associated with increased biomass. The total P accumulation of *S. nigrum* increased by 25.62 % with the addition of AMF under Cd stress (Fig. 1). However, negative effects of AMF and intercropping showed in the P uptake of rice (Fig. 1b). AMF treatment significantly decreased the shoot P concentration of the target rice, by 22.03 % and 14.13 % respectively in the Cd0 and Cd1 groups (Fig. 1d). However, AMF treatment enhanced the P uptake rate of *S. nigrum* by 1.92- and 1.76-fold in the Cd0 and Cd1 groups compared to the no-AMF treatment, but had little effect on the P uptake rate of rice (Fig. 1f). The ANOVA analysis indicated that the AMF treatment ($P < 0.05$) had a considerable effect on the total P uptake rate of *S. nigrum* and the shoot P concentration of upland rice.

3.2. Plant Cd accumulation and Cd uptake rate

The results indicated that the addition of AMF significantly increased the total Cd accumulation of *S. nigrum* shoots by 25.37 % (Fig. 2a). AMF treatment markedly reduced the Cd accumulation in rice shoots and roots by 45.18 % and 55.54 %, respectively (Fig. 2b) but increased the total Cd extracted by the intercropping system (by 18.93 %). Interestingly, AMF increased the Cd uptake rate of *S. nigrum* roots but lowered the Cd uptake efficiency of rice roots (Fig. 2de). The ANOVA analysis indicated that Cd treatment ($P < 0.01$) markedly affected the Cd accumulation and Cd uptake rate in both *S. nigrum* and rice plants. AMF

addition only influenced the Cd accumulation of *S. nigrum* shoots and the total Cd extraction by the intercropping system ($P < 0.01$).

3.3. Cd and P concentration in the root-soil interface

The rhizo-box experiment served as a further approach to assess the in situ characterization of soil Cd and phosphorus P levels. As depicted in Fig. 3, the in situ laser ablation-inductively coupled plasma-mass spectrometry (LA-ICP-MS) analysis revealed that the presence of AMF significantly diminished the concentrations of Cd and P in the rhizosphere and on the root surface of upland rice. This indicates that AMF could play a role in reducing the Cd bioavailability. Furthermore, the experiment highlighted that AMF inoculation exerted minimal influence on the Cd content of *S. nigrum*, suggesting that the soil Cd availability is less susceptible to mycorrhizal influence. Conversely, the inoculation did enhance the availability of P at the root-soil interface of *S. nigrum*, implying a positive effect on P nutrition. Moreover, the intercropped *S. nigrum* exhibited higher Cd and P contents compared to the target rice.

3.4. GRSP content and DTPA-Cd concentration in the rhizosphere

AMF inoculation had been shown to enhance the content of glomalin-related soil protein (GRSP) in intercropping systems. In comparison to monoculture system, the extracellular (EE)-GRSP and total (T)-GRSP contents were found to increase by 36.47 % and 19.01 %, respectively (Fig. 4a). Furthermore, an analysis of the diethylenetriaminepentaacetic acid (DTPA)-extractable cadmium (Cd) content in the rhizosphere of intercropped rice revealed that the mycorrhizal treatment substantially decreased the DTPA-Cd content by 53.52 % relative to the monoculture system (Fig. 4b). This reduction in DTPA-Cd content implied a potential role of AMF in mitigating the bioavailability and toxicity of heavy metals in the soil environment. Correlation

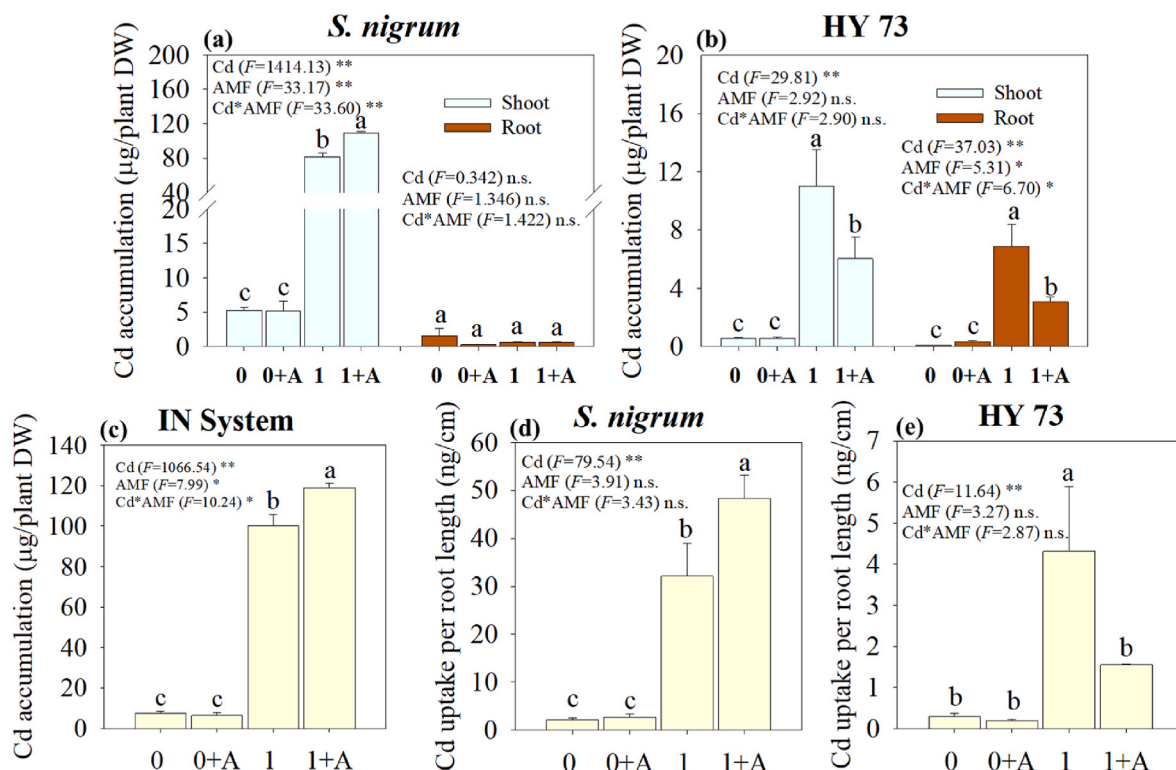


Fig. 2. Cd accumulation and Cd uptake rate of interplanted *S. nigrum* and upland rice. (a) Cd accumulation of *S. nigrum* shoot and root; (b) Cd accumulation of upland rice shoot and root; (c) Total Cd accumulation of the intercropping system; (d) Cd uptake rate of *S. nigrum* root; (e) Cd uptake rate of rice root. +A-with AMF. 0-without Cd addition (control), 1- Cd level with 1 mg/kg. Each value is the mean (\pm SE) of three replicates. Different lowercase letters show statistically significant differences among different treatments ($P < 0.05$).

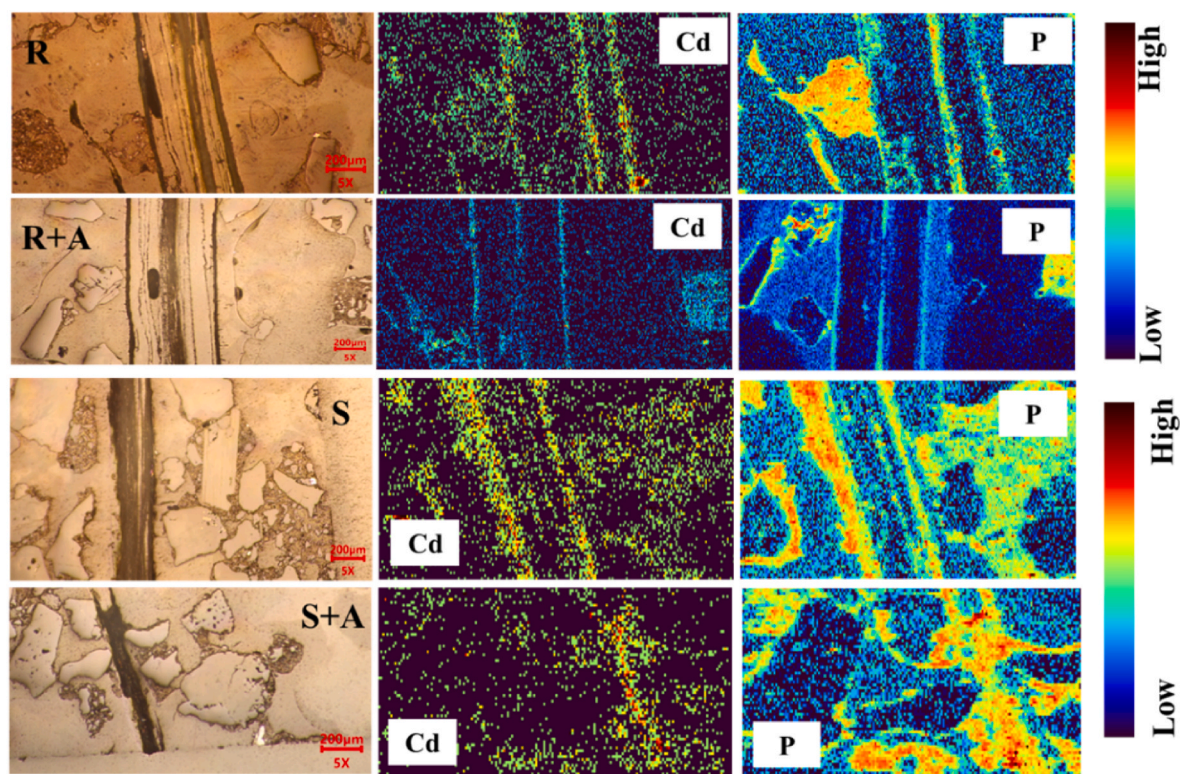


Fig. 3. Cd and P in situ analysis of roots-soil interface mediated by AMF of interplanted *S. nigrum* and upland rice. R-rice (HY 73), S-*S. nigrum*, +A-with AMF inoculation.

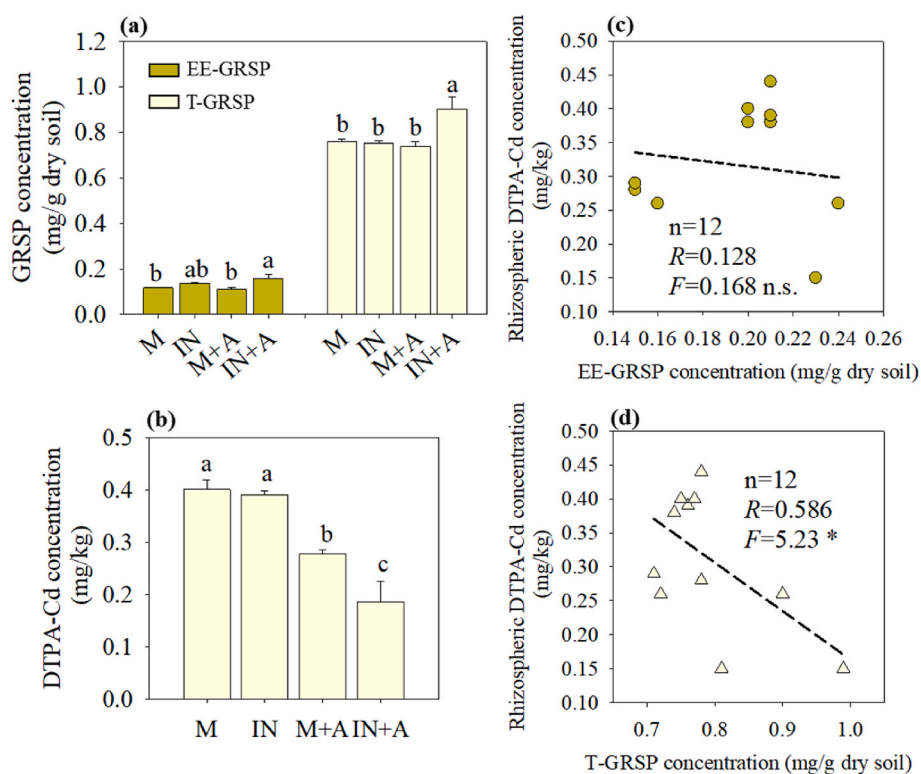


Fig. 4. The GRSP content and DTPA-Cd concentration of intercropped upland rice. (a) The EE- and T-GRSP content in rhizospheric soil; (b) the DTPA-Cd concentration in rhizospheric soil; (c) the relationship between the DTPA-Cd concentration and the EE-GRSP content; (d) the relationship between the DTPA-Cd concentration and the T-GRSP content. M-monoculture; IN- intercropping; +A-with AMF inoculation. Each value is the mean (\pm SE) of three replicates. Different lowercase letters show statistically significant differences among different treatments ($P < 0.05$).

analysis demonstrated a significant positive correlation between the concentration of DTPA-extractable Cd and the total GRSP concentration ($P < 0.05$; Fig. 4d), indicating that higher GRSP concentrations may be associated with lower bioavailable Cd levels. However, no significant correlation was observed between the EE-GRSP concentration in the soil and the DTPA-Cd concentration ($P > 0.05$), suggesting that the extracellular fraction of GRSP may not be as closely linked to Cd bioavailability as the total GRSP content. These findings underscored the potential of AMF inoculation in modifying soil properties and reducing the environmental risk associated with heavy metal contamination, likely through the mediation of GRSP.

3.5. The microbial structure of the intercropping system

The near-full-length bacterial 16S rRNA gene (V1-V9) was amplified using PCR and sequenced using the Illumina MiSeq platform, yielding a total OUT number of 2388 for further analysis (Figs. S3 and S4). The alpha diversity of the bacterial communities, as assessed by the Shannon, Chao 1, and ACE indices, was significantly higher in the mycorrhizal rhizosphere samples compared to the non-mycorrhizal rhizosphere samples in this intercropping system ($P < 0.05$; Table S1). This observation indicates that the introduction of AMF resulted in an increase in the richness of the rhizosphere microbial communities. The PCoA based on the Binary_jaccard distance of bacterial communities (OUT level) of *S. nigrum* and upland rice demonstrated that the AMF inoculation was the dominant factor influencing the similarity of the rhizosphere bacterial communities in the intercropping system of upland rice and *S. nigrum* (Fig. 5 ab), while monoculture or intercropping

patterns did not affect the structural similarity of the bacterial community within this system (Fig. S5). These findings highlight an important role of mycorrhizal associations in shaping the composition and structure of the rhizosphere microbiome in this study.

Proteobacteria (37.13–49.59 %), Bacteroidota (14.73–24.11 %), Gemmatimonadota (9.88–16.49 %), Actinobacteriota (2.50–6.12 %) and Verrucomicrobiota (3.24–4.81 %) were the five dominant bacterial phyla across all treatments (Fig. 5c). Compared with the no-AMF treatment, AMF inoculation increased the abundance of Gemmatimonadota (by 40.08 % and 31.25 %), Acidobacteriota (by 58.26 % and 54.31 %) and Firmicutes (by 31.80 % and 46.70 %) for upland rice and *S. nigrum*, respectively, while the addition of AMF decreased the abundance of Proteobacteria (by 15.86 % and 25.13 %), Bacteroidota (by 15.59 % and 26.55 %) and Verrucomicrobiota (by 32.64 % and 12.00 %) for upland rice and *S. nigrum*, respectively.

The relative abundance of *Ramlibacter*, *Flavisolibacter*, and *Gemmatimonas* accounted for the highest proportion of genera, totaling 32.27 % and 25.93 % for upland rice and *S. nigrum* (Fig. 5d), respectively. The abundance of *Flavisolibacter*, *Ramlibacter*, and *Roseisolibacter* increased by 31.18 %, 65.06 %, and 78.17 %, respectively, for upland rice with AMF inoculation, but AMF reduced the relative abundance of *Massilia* and *Ohtaekwangia* by 40.96 % and 56.21 %, respectively. For *S. nigrum*, AMF treatment increased the relative abundance of *Ramlibacter*, *Roseisolibacter*, and *Bacillus* by 54.51 %, 60.31 %, and 63.08 %, respectively, and decreased the relative abundance of *Flavisolibacter*, *Massilia*, *Ohtaekwangia*, and *Cupriavidus* by 37.94 %, 54.12 %, 28.69 %, and 58.66 %, respectively.

The genera *Ramlibacter*, *Flavisolibacter*, and *Gemmatimonas* were

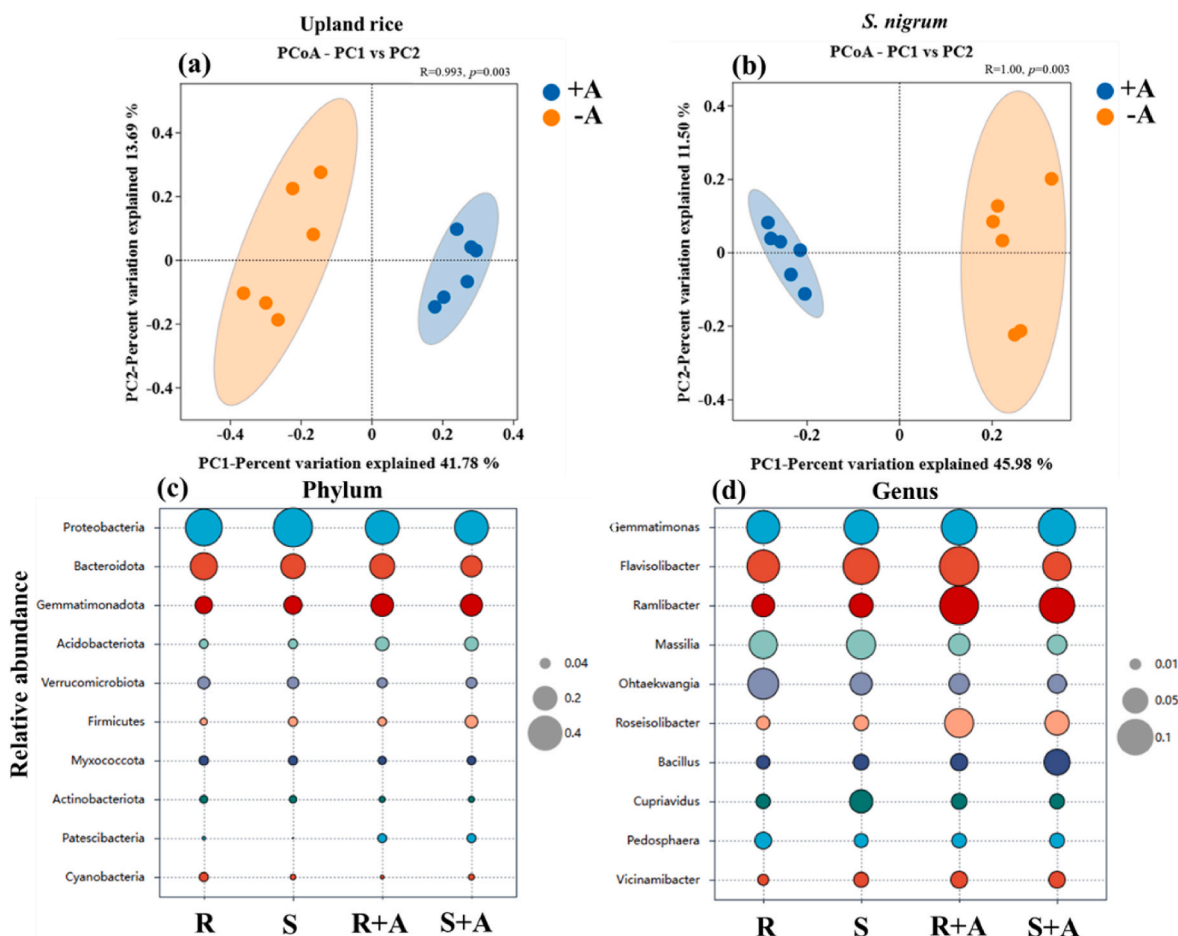


Fig. 5. β -analysis (PCoA plot) of bacterial genus of upland rice (a) and *S. nigrum* (b), and the relative abundances of dominant bacteria (top-10) at the phylum (c) and genus (d) level in the intercropping system. R-rice (HY 73), S-*S. nigrum*, +A-with AMF inoculation.

dominant in this intercropping system, constituting the highest relative abundances, which were totaling 32.27 % for upland rice and 25.93 % for *S. nigrum* (Fig. 5d). Inoculation with AMF in upland rice led to significant increases in the relative abundances of *Flavisolibacter* (31.18 %), *Ramlibacter* (65.06 %), and *Roseisolibacter* (78.17 %), while it decreased the relative abundances of *Massilia* (40.96 %) and *Ohtaekwangia* (56.21 %). Conversely, for *S. nigrum*, AMF treatment resulted in elevated relative abundances of *Ramlibacter* (54.51 %), *Roseisolibacter* (60.31 %), and *Bacillus* (63.08 %), alongside reductions in *Flavisolibacter* (37.94 %), *Massilia* (54.12 %), *Ohtaekwangia* (28.69 %), and *Cupriavidus* (58.66 %). These findings suggested that AMF inoculation exerted genus-specific effects on the relative abundance of microbial communities in both upland rice and *S. nigrum*.

4. Discussion

4.1. Effect of AMF inoculation on Cd and P uptake pattern of intercropping system

Many studies have proved that the intercropping of hyper-accumulators and crops can decrease the heavy metal uptake in the target crop while increasing the acquisition of heavy metals in hyper-accumulators. Examples include intercropped combinations of *Pak choi* and *Sedum* or fava bean and *Sedum* with inoculated endophytes (Ma et al., 2020; Tang et al., 2020), or intercropped *Pteris vittata*/*Morus alba* (Wan et al., 2017) and *S. nigrum*/eggplant (Tang et al., 2017). However, research on the remediation of heavy-metal-contaminated soil using AMF-mediated hyperaccumulators and crops in an intercropping system has only gradually gained attention in recent years, and the underlying mechanisms are still not fully understood. In particular, there is a lack of research on how to balance heavy metal uptake and nutrient absorption. Most studies have focused on how AMF affects the process of heavy metal uptake in these systems. For example, the addition of AMF (*Glomus versiforme*) reduces Cd accumulation in upland rice (especially the grains) and increases Cd uptake by intercropped *S. calendulacea* (Lei et al., 2021). Besides, *Glomus caledonium* inoculation increases the P acquisition of intercropped *Sedum alfredii* and upland Kangkong, while a dilution effect results in lower Cd uptake by Kangkong (Hu et al., 2013). Other research also shows that AMF stabilizes Cd in the soil and enabled the safe production of soybean grains when intercropped with *S. nigrum* (Wang et al., 2025). In the present study, AMF enhanced the total Cd accumulation of the intercropping system under moderate Cd stress and reduced the Cd uptake in the target rice but increased the Cd accumulation of *S. nigrum*. These results were consistent with other studies mentioned above. Notably, the addition of AMF markedly reduced the Cd availability, Cd uptake rate, and Cd accumulation in rice parts of the intercropping system. These results were consistent with some previous studies of AMF-upland rice systems (Chen et al., 2019).

Generally, the AMF inoculation has access to nutrient patches (particularly of P) outside the rhizosphere by producing an extensive network of fine hyphae (Bhantana et al., 2021). Plants deliver carbon in the form of sugars and lipids to AMF in return for nutrients; in exchange, AMF supplies nutrients (particularly P) for the host plant (Edlinger et al., 2022; Smith and Read, 2008). Literature indicates that AMF inoculation also contributes to the enhanced acquisition of phosphorus by intercropped plants. For example, inoculation with AMF can improve the phosphorus use efficiency in an intercropped maize with soybean (Song et al., 2021), or increase the phosphorus concentration in intercropped alfalfa (Zhang et al., 2018). Other studies have also found that AM fungi can promote the growth and phosphorus content of intercropped upland rice and the Cd-hyperaccumulator (*Sphagneticola calendulacea*) in Cd-contaminated soil (Lei et al., 2021). However, these AMF reciprocal strategies primarily rely on the allocation of aboveground photosynthetic carbon resources in plants (Bever et al., 2009; Zheng et al., 2015). Additionally, the cost of regulating resource allocation may limit plants from efficiently regulating these strategies (such as plant root growth,

mycelial growth, exudation release, core microbial recruitment, etc.) simultaneously (Lambers et al., 2006; Wen et al., 2022). This implies that the intercropping plants may have different Cd and P allocation trade-offs. In the present study, AMF inoculation significantly increased P uptake of intercropped *S. nigrum*, consistent with the above results. Conversely, AMF decreased the shoot P uptake of the target rice by 22.03 % and 14.13 % in the Cd0 and Cd1 groups, respectively (Fig. 2). These P uptake properties may have been caused by reduced P uptake rate and lowered P availability at the root-soil interface of the intercropped rice. Besides, Zhang et al. (2018) indicated that AMF decreases the P concentration of the intercropped pepperweed, and provides protective effects of AMF on pepperweed against As contamination. While AMF increases P uptake in the neighboring alfalfa, by competing for P absorption with pepperweed (Zhang et al., 2018). We speculated that the lower P content in the target rice might be caused by the interspecific competition between the two intercropped plants (Liao et al., 2023), as the external hyphae or roots from *S. nigrum* could reach the rhizosphere of upland rice and take away P in soil. Another possible reason was that AMF would preferentially allocate more phosphorus to plants that provide more carbon resources for AMF (Bever et al., 2009). Taken together, these results indicated that AMF probably had significantly different strategies for obtaining P and Cd in the hyper-accumulator *S. nigrum* and rice intercropping system. That is, AMF tended to improve the nutrient (especially P) acquisition ability of the intercropped hyperaccumulating plants by promoting growth, which increased the heavy metal extraction rate while strengthening the Cd-defending capability of the target rice.

4.2. Cd binding effect of intercropped rice root

The secretion of root exudates is one of the important mechanisms to modify the rhizosphere to promote the heavy metal uptake of plants (Rajniak et al., 2018). In the present study, the EE-GRSP and T-GRSP content in the rhizosphere soil of upland rice was highest in the intercropping treatment with mycorrhizal addition. The T-GRSP content in the soil of upland rice was significantly negatively correlated with the Cd concentration in the aboveground part of upland rice, while the T-GRSP content in the rhizosphere was significantly negatively correlated with the DTPA-Cd content in the rhizosphere (Fig. 4). GRSP is a stable and persistent protein produced in large quantities by mycorrhizal fungi and typically divided into two parts: EE-GRSP and T-GRSP (Jia et al., 2016). GRSP can not only sequester soil carbon but may also serve as an important reservoir of organic nitrogen in the soil and stabilize heavy metals such as Pb, Cd, Mn, etc. in soil/water bodies (Ji et al., 2019; Malekzadeh et al., 2016). Some studies have found that GRSP can serve as an indicator for land-sea connectivity, feedback terrestrial nutrient transport, and as an iron-rich carrier to promote carbon fixation (Wang et al., 2019). Other research also indicates that *Pteris vittata*-AMF symbiosis presents a higher contribution to sequestering most heavy metals by T-GRSP content in the contaminated sites (Qiu et al., 2022). Therefore, the reduction in Cd uptake by rice could be attributed to an AMF-induced increase in GRSP content, which bound Cd in the rhizosphere and limited its bioavailability. This result was consistent with other research that indicated the stabilizing effect of T-GRSP on Cd in the soil of this system (Jia et al., 2016). Gillespie et al. (2011) found early that GRSP may be a partner of sulfiredoxin and that it contains a large amount of soil-related heat-stable protein and non-mycorrhizal protein, lipid, and humus (Gillespie et al., 2011; Jia et al., 2016). Therefore, in the present study, the T-GRSP content could better characterize the relationship between GRSP content and soil Cd than the EE-GRSP content could.

4.3. Microbial structure of the intercropping system

The microbial community structure in soil affects plant growth and nutrient cycling (Zechmeister-Boltenstern et al., 2015). The addition of

AMF can regulate the rhizospheric microbial composition and foster certain beneficial microbes to enhance plant tolerance to heavy metal stress (Zhao et al., 2023). For example, Chen et al. (2019) found that *R. intraradices* could significantly increase Actinomycetes in the rhizosphere soil of rice (Chen et al., 2019). Another study also indicated that AMF significantly enriches more beneficial rhizosphere bacteria (mainly *Rhodobacter*, *Archangium*, and *Longimicrobium*) that tolerate heavy metals and facilitate *Astragalus adsurgens* growth (Li et al., 2023). In the present study, mycorrhizal addition significantly influenced the rhizospheric microbial (bacterial) communities of *S. nigrum* and upland rice in the intercropping system (Table S1). The PCoA analysis also indicated that mycorrhizal treatment significantly altered the soil bacterial structure in the rhizosphere of *S. nigrum* and upland rice (Fig. S3). The AMF addition mainly increased the relative abundance of *Gemmatimonadota* and *Acidobacteriota* but decreased the abundance of *Proteobacteria* and *Bacteroidota* (Fig. 5a). Other studies have also found similar results, where AMF inoculation can alter the rhizosphere microbial community structure and abundance in intercropping systems. Recent study indicated that AMF (*Glomus mosseae*) promotes recruitment of metal-resistant microbial community (including *Parvibaculum*, *Massilia*, and *Alistipes*), which help reduce Cd migration in *Medicago sativa* (Wang et al., 2023a). Other research also indicates that AMF inoculation reduces the soil Cd risk in an intercropping system of soybean and *S. nigrum*, by regulating the soil microbial genera (Wang et al., 2025).

AMF inoculation notably enhanced the relative abundance of *Ramlibacter*, *Bacillus*, and *Roseisolibacter* of *S. nigrum* in the intercropping system but lowered the bacterial abundance of *Flavisolibacter*, *Massilia*, *Ohtaekwangia* and *Cupriavidus* (Fig. 5b). Many reports have indicated that *Gemmatimonas*, *Ramlibacter* and *Bacillus* have phosphate-solubilizing and plant growth-promoting traits (Qin et al., 2023; Rawat et al., 2021). *Gemmatimonas*, an essential genus for phosphate solubilisation, can dissolve inefficient phosphorus and convert it for plant growth (Takaichi et al., 2010). Chen et al. (2022) also found that *Ramlibacter* promotes P uptake by ryegrass. *Roseisolibacter* also belongs to the Gemmatimonadetes phylum (Pascual et al., 2018), indicating that AMF strongly influenced the soil P function in the intercropping system in Cd-contaminated soil, especially for the intercropped *S. nigrum*.

Massilia is a rhizospheric microorganism that increases the metabolic activity of the roots. *Massilia* promotes the metabolic activity of ryegrass roots, thereby accelerating their absorption of heavy metals (Chen et al., 2022). Another study also proves that *Massilia* is a key bacterial genus to promote maize growth performance (Wang et al., 2024a). *Ohtaekwangia* (members of the Bacteroidetes phylum) have also been characterized as a contamination-tolerant species (Brereton et al., 2020; Yoon et al., 2011). *Flavisolibacter* has been identified as the controlling bacteria in influencing the degradation of pollutants, suggesting the possible function of pollutant stress (Wang et al., 2024b). Additionally, *Cupriavidus* has shown a strong tolerance to heavy metals (e.g. Cu, Pb, Zn, Cd) and is used for heavy metal wastewater (Shi et al., 2023).

These results suggested that AMF addition mainly increased P-solubilizing bacteria for the intercropped *S. nigrum* but decreased bacteria with the function of heavy metal tolerance. Interestingly, AMF inoculation particularly enhanced the relative abundance of *Flavisolibacter*, *Ramlibacter*, and *Roseisolibacter* in rice in this intercropping system but lowered the abundance of *Massilia* and *Ohtaekwangia* (Fig. 5). These results implied that AMF addition recruited microbes both with a P function and with heavy metal tolerance for upland rice; however, the *Flavisolibacter* abundance was much higher than that of *S. nigrum*, suggesting the target rice had higher metal tolerance. Though AMF would recruit a similar bacterial composition for both *S. nigrum* and upland rice, the species proportions were different for these two plants. This implied possible different function preferences of *S. nigrum* and intercropped upland rice. However, these results were obtained at one sampling stage only; the effect of AMF on the recruitment of different microbes in intercropping systems still needs further research.

5. Conclusions

The results demonstrated that AMF mediated the Cd and P allocation strategy of the Cd hyperaccumulator and neighboring rice in Cd-contaminated soil. The addition of AMF increased Cd and P accumulation by *S. nigrum* and reduced the Cd uptake of the neighboring rice. The addition of AMF also increased GRSP content in the rhizosphere of the target rice, which was responsible for the lower available Cd content. In-situ LA-ICP-MS analysis demonstrated the strong hindering effect of AMF on the Cd content of the rice rhizosphere and root surface, as well as the increase in total P accumulation and the P and Cd uptake rate per root length of *S. nigrum* upon AMF addition. Additionally, AMF inoculation reshaped the microbial structure in the intercropping system, especially the abundance of phosphate-solubilizing bacteria *Ramlibacter*, *Bacillus*, and *Roseisolibacter* for *S. nigrum*. AMF stimulated the intercropped *S. nigrum* to acquire more P than the neighboring rice, thereby providing a resource acquisition effect but preferentially strengthening the rice-defending effect in the intercropping system. This strategy holds economic promise by improving rice marketability through reduced Cd uptake and enabling profitable Cd recovery via *S. nigrum*. Enhanced phosphate-solubilizing microbes lower fertilizer dependency, cutting costs and environmental impacts. These benefits position it as a cost-effective solution for Cd-contaminated farmlands. However, field trials are critical to validate scalability, as microbial dynamics and Cd-remediation efficiency may vary under diverse soil and climatic conditions. Long-term studies are also needed to assess the stability of AMF-mediated benefits and cost-effectiveness across different agricultural systems.

CRediT authorship contribution statement

Xu Yang: Writing – original draft, Methodology, Investigation, Funding acquisition, Conceptualization. **Qiuyu Chen:** Methodology, Investigation. **Zhuomin Jiang:** Investigation. **Wenzhen Chen:** Methodology, Investigation. **Tuantuan Cui:** Software, Methodology. **Bohan Wu:** Software, Conceptualization. **Huashou Li:** Funding acquisition, Conceptualization. **Rongliang Qiu:** Writing – review & editing, Funding acquisition, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.envpol.2025.126382>.

Data availability

Data will be made available on request.

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Salinity elevates Cd bioaccumulation of sea rice cultured under co-exposure of cadmium and salt

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ABSTRACT

Salt-tolerant rice (sea rice) is a key cultivar for increasing rice yields in salinity soil. The co-existence of salinity and cadmium (Cd) toxicities in the plant-soil system has become a great challenge for sustainable agriculture, especially in some estuaries and coastal areas. However, little information is available on the Cd accumulating features of sea rice under the co-stress of Cd and salinity. In this work, a hydroponic experiment with combined Cd (0, 0.2, 0.8 mg/L Cd²⁺) and saline (0, 0.6%, and 1.2% NaCl, W/V) levels and a pot experiment were set to evaluate the Cd toxic risks of sea rice. The hydroponic results showed that more Cd accumulated in sea rice than that in the reported high-Cd-accumulating rice, Chang Xianggu. It indicated an interesting synergistic effect between Cd and Na levels in sea rice, and the Cd level rose significantly with a concomitant increase in Na level in both shoot ($r = 0.54$, $p < 0.01$) and root ($r = 0.66$, $p < 0.01$) of sea rice. Lower MDA content was found in sea rice, implying that the salt addition probably triggered the defensive ability against oxidative stress. The pot experiment indicated that the coexistent Cd and salinity stress further inhibited the rice growth and rice yield, and the Cd concentration in rice grain was below 0.2 mg/kg. Collectively, this work provides a general understanding of the co-stress of Cd and salinity on the growth and Cd accumulation of sea rice. Additional work is required to precisely identify the phytoremediation potential of sea rice in Cd-polluted saline soil.

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Introduction

Cd is widespread harmful heavy metal in the environment that possesses high mobility and toxicity to the living organisms (Satarug et al., 2010; Sun et al., 2022; Zhang et al., 2014). It

is reported that about 2.79×10^9 m² of agricultural soils were polluted with Cd in China (Liu et al., 2015). Additionally, agricultural production is facing increasingly urgent challenges from salt intrusion due to global warming, ice melt, and rising sea levels (Thu et al., 2017). The global saline and sodic soils cover 397 million and 434 million hectares, respectively,

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and 19.5% of the irrigated lands have been affected by salt (FAO, 2016). Literature suggests that many arid and semi-arid areas, and coastal areas in the world are affected by simultaneous salt stress and heavy metal pollution (Liang et al., 2017). For instance, in the eastern coastal areas of China, the rapid industrial development and the use of fertilizers have introduced a large number of heavy metals, such as Cd, into the saline tidal lands and the aquatic system (Green-Ruiz et al., 2008; Han et al., 2017), thereby causing serious problems in environmental management and food production (Han et al., 2012). An increasing trend of salinity and heavy metal contamination in the coastal area demand research on some particular plants which can help in overcoming these compounding problems (Bai et al., 2019). But hyperaccumulators are generally not used for phytoremediation in these polluted areas because they are sensitive to salinity (Helal et al., 1996; Lefèvre et al., 2009). Therefore, halophytic species are regarded as the appropriate plants as they can survive under the combined heavy metal and salinity stress (Kouhi and Moudi, 2020).

Global food demand is likely to double by 2050 to meet the growing demand and climate change (Tilman et al., 2011). Salt-tolerant rice cultivars (*Porteresiacoarctata* Tateoka) have been considered to be one of the key strategies to increase rice production, and this rice cultivar can grow in marginal soils and adverse environments, such as seawater submerged, saline-alkaline soil near the coastal region (Thu et al., 2017). For example, the sea-rice 86 (SR 86) cultivar is estimated to keep an acceptable average yield of 2,250 kg/ha though growing in extreme environments (Chen et al., 2017). Literature documented that cultivating rice is also one of the efficient ways to improve saline soils, as rice plants can increase the soil nutrient contents and diversify soil microorganisms (Xu et al., 2020). However, our previous study suggests that the sea rice (HH12) possesses relatively high Cd accumulative features compared with another two rice cultivars (relatively low and high Cd-accumulating rice cultivars), and the shoot Cd content in HH12 is as high as 168.99 mg/kg with hydroponic culture (Yang et al., 2019). Besides, the relative expression levels of the key genes related to Cd uptake (*Nramp5*) and Cd translocation (*HMA2*) in sea rice roots were much higher than those of the low-Cd accumulating rice cultivar with Cd stress (Appendix A Fig. S1). Notably, multiple studies indicate that salinity significantly enhances the heavy metal mobility in the soil and the accumulation in plant tissues (Wang et al., 2019; Zahedifar and Moosavi, 2020). Cd also exhibits higher ecological risk and mobility in soil with a salinity effect (Zhao et al., 2013). While little information is concerned about the Cd accumulating features (especially the Cd concentration in rice grain) of the salt-tolerance rice cultivar under the co-existent Cd and salinity stress. Therefore, the objectives of this study were: (1) to investigate the Cd accumulating features of sea rice under co-stress of Cd and salinity; (2) to identify the interaction of Cd and salinity on the Cd uptake by sea rice; and (3) to assess the toxic risks of Cd on sea rice.

1. Materials and methods

1.1. Plant materials and culture

The rice cultivars used in this study were Hai Hong 12 (HH12, salt tolerance: 0.6% soil salinity) and one reported high-Cd rice cultivar, Chang Xianggu (Ishikawa et al., 2006; Xu et al., 2021), which were obtained from the Institute of Subtropical Agriculture of Chinese Academy of Sciences. The rice seeds were surface-sterilized by soaking in 10% H₂O₂ for 15 min, then rinsed with deionized water and placed in containers with moistened sands for germination. Seed germination was conducted in a temperature-controlled incubator (28/25 °C, 16/8 hr, day/night) with a relative humidity of 70% for about 30 days. These seedlings were further placed in 250 ml beakers for 7 days, containing 150 mL of 50% Yoshida's solution, prepared in deionized water (Yang et al., 2019). The pH of the solution was adjusted to pH 5.0–5.20. The macroelement compositions of Yoshida's stock solution were composed of (in mol/L) 1.14 N, 0.26 P, 0.41 K, 1.13 Mg, 0.80 Ca, 1.54 S, 15.83 Si; and the microelement compositions were composed of (in mmol/L) 28.49 Fe, 6.43 Mn, 15.11 B, 0.12 Cu, 0.12 Zn, 0.06 Mo (Yoshida, 1976). All the beaker walls were covered with black paper to avoid exposure of light to the root.

1.2. Soil preparation

The tested soil was collected from the experimental field of the Ecology Department, South China Agricultural University (23°16'N, 113°37'E). The soil was dried naturally and sifted by a 2 mm sieve, without any soil gravel, leaves, and other debris. The tested soil was thoroughly blended with the CdCl₂·5/2H₂O and NaCl for a month to stay homogeneous before the transplanting of rice seedlings (Yang et al., 2021). The pH of the tested soil was 7.02, containing 19.60 g/kg organic matter content, 0.09 g/kg available N, 0.17 g/kg available P, and 0.12 g/kg available K.

1.3. Experimental design

1.3.1. Hydroponic experiment

The hydroponic experiment (30 days) was conducted in the greenhouse of the College of Agriculture, South China Agricultural University (23°16'N, 113°37'E) to observe the salinity effect on the Cd uptake in sea rice (HH12) and the reported high-Cd accumulating rice (Chang Xianggu). Uniform and healthy seedlings of each rice cultivar were transplanted to 100% Yoshida's solution. Different treatments were organized with three replications in randomized factorial design as Cd 0 mg/L + (0%, 0.6%, and 1.2% NaCl, W/V); Cd 0.2 mg/L + (0%, 0.6%, and 1.2% NaCl, W/V); Cd 0.8 mg/L + (0%, 0.6%, and 1.2% NaCl, W/V), respectively. The Cd levels were supplied as CdCl₂·5/2H₂O. The different saline levels were determined based on pre-experiment and were supplied as NaCl (W/V). The salinity and conductivity features of each treat-

Table 1 – The salinity and conductivity features of the hydroponic solution.

Treatments	Na0Cd0	Na0Cd1	Na0Cd2	Na1Cd0	Na1Cd1	Na1Cd2	Na2Cd0	Na2Cd1	Na2Cd2
Salinity (‰)	0.6	0.6	0.6	1.2	1.2	1.2	1.8	1.8	1.8
Conductivity (mS/cm)	1.2	1.2	1.2	2.29	2.28	2.30	3.39	3.36	3.4
Na0= no extra NaCl added (0% Na), Na1=extra 0.6% NaCl added (W/V), Na2=extra 1.2% NaCl added (W/V); Cd0=0 mg/L Cd, Cd1=0.2 mg/L Cd, Cd2=0.8 mg/L Cd. E.g., Na0Cd0 = 0% NaCl, 0 mg/L Cd, the follow-ups were similar.									

ment were showed in Table 1. Each pot (diameter = 21.5 cm, height = 14.3 cm) contained 5 L nutrient solution. Each treatment had 3 replicates with two rice seedlings for each pot. Yoshida's solution was renewed every week, and pH was adjusted to 5.0–5.2 every three days with potassium hydroxide (KOH) or hydrochloric acid (HCl).

1.3.2. Pot experiment

A pot experiment was used to observe the growth and Cd accumulating performance of HH12 and Chang Xianggu with simultaneous Cd and salinity stress. The pot experiment started in April 2020 and was carried out in the greenhouse of the Ecology Department, South China Agricultural University (23°16'N, 113°37'E). Two uniform-sized seedlings (height: ~15 cm) of each rice cultivar were selected from the germinated seeds used for this experiment with a completely randomized design. The experimental variables were: two soil Cd concentrations (0 and 1.0 mg/kg, supplied as $\text{CdCl}_2 \cdot 5/2\text{H}_2\text{O}$) and two soil salinity [0 and 0.3% (W/W), supplied as NaCl], three replicates in each treatment. Each pot contained 4 kg of paddy soil. A compound fertilizer (15-15-15) was used to maintain the N, P, and K balance for rice growth (Yang et al., 2020). The water surface was 1–2 cm higher than the soil surface during the growth process, and HH12 and Chang Xianggu were, respectively harvested on the 111th and 90th days after transplanting.

1.4. Sampling and analysis

1.4.1. Sample harvest

Samples of the hydroponic experiment were harvested after a 30-day treatment. The rice plants of the pot experiment were collected in July (Maturity stage of Chang Xianggu, 90 days) and August (Maturity stage of HH12, 111 days), 2020, respectively. After collection, the straw and root portions of the rice plant were separated. The fresh roots of the hydroponic experiment were soaked in 25 mmol/L $\text{Na}_2\text{-EDTA}$ solution for 15 min to remove the metal ion adsorbed on the root surface and rinsed thoroughly with deionized water. Rice plants of the pot experiment were separated into rice grain (hulled grain), husk, straw, and root at the ripening stage. The plant roots were rinsed successively with tap water and distilled water to remove the attached impurities (Yang et al., 2021). The collected plant samples were oven-dried at 105 °C for 20 min and kept at 75 °C for about three days until a constant weight was attained.

1.4.2. Cd and Na determination

The dried samples of these rice plants were carefully ground in a stainless steel mill, and digested by microwave diges-

tion (CEM MARS6, USA) with the mixed $\text{HNO}_3\text{-H}_2\text{O}_2$ solution (Yang et al., 2020). Meanwhile, the quality control (CDHK-GBW(E)100349, Certified reference material for the chemical composition of rice flour) and the blank samples were generated. Total Cd content and Na content were detected by inductively coupled plasma-mass spectrometry (ICP-MS, NexION 2000, USA) and the graphite furnace atomic absorption spectrometry (AAS, Z700P, Germany). The recovery of the standard detected by ICP-MS for each element ranged between 90% and 120%. The Cd recovery rate of the reference material detected by AAS was $99.03\% \pm 4.18$. The reagents used in the experiments were analytical reagents.

1.4.2. Root parameters and MDA (Malondialdehyde) content

Another rice plant in the hydroponic experiment was selected to acquire root parameters and MDA content. After soaking in 25 mmol/L $\text{Na}_2\text{-EDTA}$ solution for 15 min, the fresh roots were scanned by a root scanner (Epson Expression 1600 pro, Model EU-35, Japan) and analyzed by WinRHIZO Reg2009 to acquire the root parameters.

The MDA contents in rice shoots were assessed following the modified procedure given by Kamran et al. (2019). The fresh shoot (0.50 g) was first carefully ground with 2 mL 10% TCA (Trichloroacetic acid) and then with another 3 mL 10% TCA for further grinding. The homogenate obtained after milling was centrifuged at 3000 r/min for 10 min, and the supernatant was used as a sample extract. 2 mL of 0.5% TBA solution was mixed with 2 mL of the supernatant obtained in the above step, and the mixed solution was put in the water bath (95 °C) for 20 min, then quickly cooled and centrifuged (3000 r/min, 10 min). The supernatant was measured with the UV-Vis spectrophotometer (TU-1901, China) at 532, 600 and 450 nm wavelengths. The control tube replaced the extract with 2 mL of ultrapure water. The result of MDA concentration was calculated as followed: $C_{\text{MDA}} (\mu\text{mol/L}) = 6.45 \times (\text{OD}_{532} - \text{OD}_{600}) - 0.56 \times \text{OD}_{450}$.

1.5. Data analysis

Experimental data were analyzed using a one-way or two-way analysis of variance (ANOVA) and the means were compared using a significant difference (Duncan) method at a 5% level (SPSS 17.0). The correlation analysis in Fig. 5 was calculated by Pearson correlation (SPSS 17.0). And figures were finished using Sigmaplot 10.0.

The calculation of transfer factors (TF) was as follows:

$\text{TF} = \text{Ctissue1}/\text{Ctissue2}$, where Ctissue1 and Ctissue2 refer to the Cd contents in the different parts of the rice plant, in mg/kg (Zhang et al., 2014).

2. Results and discussion

2.1. Plant growth, total root length, and MDA content in rice plants grown in the hydroponic culture

Both Cd and saline stress have remarkable detrimental effects on plant growth. In general, inhibition of root elongation is considered the first evidence of Cd toxicity in plants, as the cell division in the root tip and the cell elongation in the extension region are easily influenced by toxic heavy metals (He et al., 2010; Munzuruglu and Geckil, 2002; Yang et al., 2019). In addition, salinity also inhibits shoot and root development, especially root growth, morphology, anatomy, and physiology (Ibrahim et al., 2015; Lakhdar et al., 2008). Different Cd and salinity levels indicated no markable effects on the plant biomass of Chang Xianggu, but high Cd and saline stress significantly reduced shoot biomass of HH12, compared with CK (Appendix A Table S1). Additionally, the total root length of HH12 showed no significant decrement under different salinity and Cd stress (except for high Na and Cd levels). The root length of HH12 at the Na2Cd2 level decreased by 57.30%, compared with CK (Fig. 1). Based on the ANOVA analysis, the Na treatment ($p = 0.71$), Cd treatment ($p = 0.07$), and the Cd \times Na interaction ($p = 0.17$) all showed no marked effect on the total root length of HH12. The total root length of Chang Xianggu presented a similar tendency as HH12, but the total root length of Chang Xianggu was lower than HH12, which is probably due to the cultivar variation (Lu et al., 2013). And based on the multi-factor analysis, the rice cultivars ($F = 19.04$, $p < 0.01$) and Cd \times Na \times cultivar ($F = 4.16$, $p < 0.01$) indicated noticeable effects on the total root length (Appendix A Table S2).

Commonly, MDA is the main product of cell membrane lipid peroxidation, being an excellent marker of oxidative stress damage in plants (Shah et al., 2001). The content of

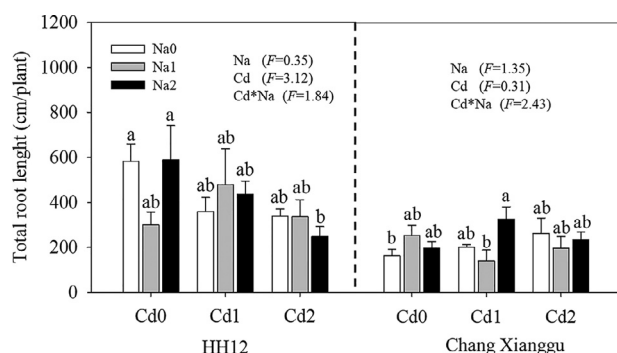


Fig. 1 – Total root length of HH12 and Chang Xianggu under different salinity and Cd stress. Na0= no extra NaCl added, Na1=extra 0.6% NaCl added (W/V), Na2=extra 1.2% NaCl added (W/V); Cd0=0 mg/L Cd, Cd1=0.2 mg/L Cd, Cd2=0.8 mg/L Cd. Lowercase letters on the bar chart indicate significant differences among different treatments ($p < 0.05$). **denotes $p < 0.01$, *denotes $p < 0.05$, the same below. Data were presented as mean \pm standard error ($n = 3$).

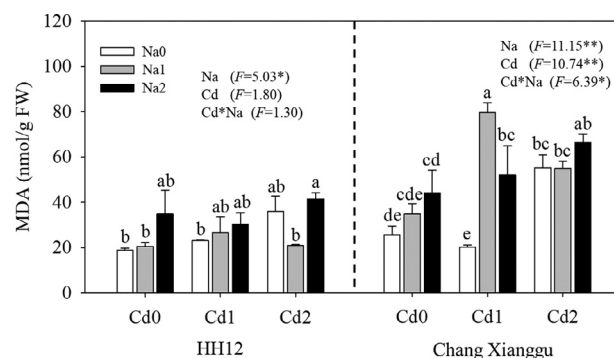


Fig. 2 – MDA content in HH12 and Chang Xianggu under different salinity and Cd stress. Lowercase letters on the bar chart indicate significant differences among different treatments ($p < 0.05$). **denotes $p < 0.01$, *denotes $p < 0.05$, the same below. Data were presented as mean \pm standard error ($n = 3$).

MDA well indicates the plant salt stress and salt tolerance (Liang et al., 2018). Additionally, salinity stress usually significantly increases the content of MDA (Taïbi et al., 2016). In this work, the highest MDA content of HH12 was found at high saline and high Cd treatment (Na2Cd2), which indicated the occurrence of oxidative stress (Shao et al., 2008). But there was no significant difference between Cd0, Cd1, and Cd2 treatments when treated with Na0, Na1, and Na2 levels in HH12 (Fig. 2). As for Chang Xianggu, the Na and Cd treatments and the Cd \times Na interaction all notably influenced MDA content in the shoot ($p < 0.01$). Besides, the high Cd level (0.8 mg/L) significantly increased the MDA content of Chang Xianggu at Na0, Na1, and Na2 levels. When compared with Chang Xianggu, lower MDA content was observed in HH12, indicating the alleviation of oxidative stress due to the combined Cd and Na stress, i.e. the addition of salinity induced effective antioxidants to Cd stress (Ibrahim et al., 2015; Khan et al., 2020). The multi-factor analysis indicated that Cd treatment, Na treatment, rice cultivars, Cd \times Na, Na \times cultivar, and Cd \times cultivar all showed marked effects on the MDA content (Appendix A Table S2).

2.2. Plant biomass of rice plants cultured with Cd-polluted saline soil

The excess heavy metal seriously destroyed plant growth, by disturbing plant physiology, inhibiting root prolongation, etc. (He et al., 2010; Ur Rehman et al., 2017). In this study, rice biomass was significantly inhibited by Cd exposure, and the combined stress of Cd and salinity further posed a negative effect on the plant biomass of Chang Xianggu and HH12. Compared with CK, the Cd stress and Cd+Na treatment markedly lowered the shoot biomass and grain yield of Chang Xianggu by 42.27% and 17.83%, and 29.21% and 70.87%, respectively. The Cd stress reduced the root, shoot, and grain biomass of HH12 by 60.72%, 42.75%, and 52.40%, respectively, compared with CK treatment. The Cd+Na group also dramatically inhibited the root, shoot, and grain biomass of HH12, by reducing 66.78%, 40.34%, and 60.93%, respectively, compared with the

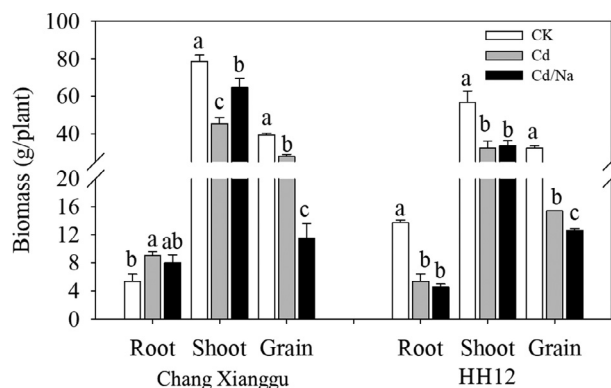


Fig. 3 – Plant biomass of HH12 and Chang Xianggu under different salinity and Cd stress. Lowercase letters on the bar chart indicate significant differences among different treatments ($p < 0.05$). Data were presented as mean \pm standard error ($n = 3$).

control (Fig. 3). These results indicated that the co-presence of Cd and salinity has more negative effects on the rice growth compared to these stresses alone, which were consistent with some previous results (Abbas et al., 2018; Hussain et al., 2014). Salinity in soil increase the Cd bioavailability by the formation of Cd-chloride complexes (Li et al., 2019), thereby aggravating the Cd toxicity to the rice plant, as the salinity and Cd stress generally decreased the plant height, root length, photosynthetic rate, and activities of antioxidant enzymes in plants (Shafi et al., 2009).

2.3. Cd concentration in rice plants with different Cd and saline levels grown in the hydroponic culture

The different Na, Cd treatments and the Cd \times Na interaction presented different patterns in the Cd concentration in HH12 and Chang Xianggu (Fig. 4). Without Cd stress or at a low Cd level, the Cd concentration in the HH12 shoot showed no differences among different Na0, Na1, and Na2 treatments. But at a high Cd level, the Cd concentrations in Na1Cd2 and Na2Cd2 shoot were notably higher than that of Na0Cd2, increasing by 15.47% and 17.98%, respectively (Fig. 4a). As for Chang Xianggu, the shoot Cd concentration presented no differences among different Na0, Na1, and Na2 treatments with or without Cd stress (Fig. 4b). Interestingly, based on the ANOVA analysis, the Na treatment ($p = 0.02$) and Cd treatment ($p < 0.01$) showed an obvious effect on shoot Cd concentration of HH12, but only the Cd treatment markedly influenced shoot Cd concentration of Chang Xianggu ($p < 0.01$).

Similarly, Na2Cd1 significantly increased the root Cd concentration of HH12, increasing by 48.92%, compared with Na0Cd1. While at a high Cd level, Na1Cd2 markedly enhanced the root Cd concentration of HH12, increasing by 50.23%, compared with Na0Cd2. As for Chang Xianggu, without Cd stress or at a low Cd level, the Cd concentration in the root showed no differences among different Na0, Na1, and Na2 treatments. Based on the ANOVA analysis, the Na treatment ($p = 0.03$), Cd treatment ($p < 0.01$), and the Cd \times Na interaction ($p < 0.01$) all

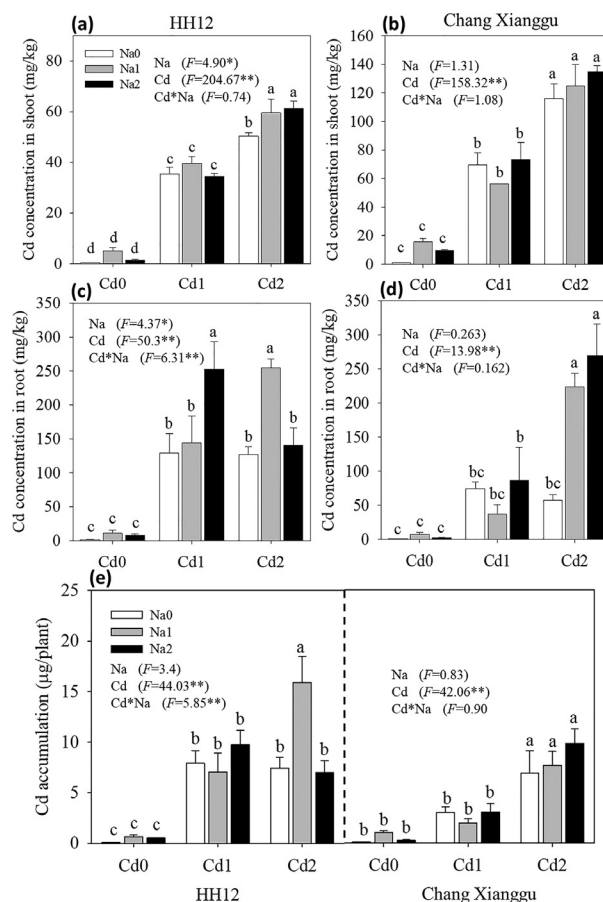


Fig. 4 – Cd concentration in shoot and root of HH12 (a,c) and Chang Xianggu (b,d) and their Cd accumulation (e) under different salinity and Cd stress. Lowercase letters on the bar chart indicate significant differences among different treatments ($p < 0.05$). **denotes $p < 0.01$, *denotes $p < 0.05$, the same below. Data were presented as mean \pm standard error ($n = 3$).

showed a significant effect on root Cd concentration of HH12, but only Cd treatment markedly affected root Cd concentration of Chang Xianggu ($p < 0.01$).

Noticeably, the Cd bioaccumulation in HH12 was much higher than that of Chang Xianggu at a low Cd level, which was 2.64, 3.59, and 3.19 fold that of Chang Xianggu, respectively at Na0, Na1, and Na2 treatments (Fig. 4e). The highest Cd accumulation was found in HH12 under Na1Cd2 treatment (15.90 $\mu\text{g/plant}$), which was 2.07 times that of Chang Xianggu. Additionally, Cd treatment ($p < 0.01$) and the Cd \times Na interaction ($p < 0.01$) significantly influenced Cd accumulation in HH12, while only Cd treatment affected Cd accumulation in Chang Xianggu ($p < 0.01$).

Works of the literature suggest that Cd accumulation in the plant would be increased in Cd-contaminated soil with saline stress, resulting from the formation of a Cl^- -Cd complex in soil (Dutta et al., 2019; Khan et al., 2020; Weggler-Beaton et al., 2000; Zhao et al., 2013). For example, Li et al. (2019) demonstrated that chlorine application increases the Cd concentration in soil pore water, and enhances the Cd concentration

in the rice tissues. Besides, [Shao et al. \(2008\)](#) showed that rice plants pretreated with NaCl for 5 days induce higher Cd concentrations in both roots and shoots of two rice genotypes. However, the Cd, Na, and Cl forms were ionic states in our hydroponic culture, which differed from the above results. These hydroponic results showed the NaCl effect on Cd uptake independently of Cd bioavailability, which implied that the root cell membrane damage might thus contribute to the non-selective entry of Cd in the solution condition ([Lutts and Lefèvre, 2015](#)). Besides, a longer total root length ([Fig. 1](#)) and a larger root surface area (data not showed) were observed in HH12 than that of Chang Xianggu, indicating that HH12 probably had a larger Cd absorption area in this work ([Huang et al., 2015](#)). Furthermore, the heavy metal detoxification and salt tolerance mechanisms of rice probably share some common characteristics, including the antioxidant systems, the intracellular compartments, and the osmoprotectants in osmotic adjustment systems ([Fu et al., 2011; Yang et al., 2019](#)). [Xu et al. \(2010\)](#) also find that low salt supplementation reduced the reactive oxygen species level in Cd-stressed roots, including the increment of the proline content and glutathione. This research revealed that the Cd response to plant salinity was associated with an increase in phytochelatin levels and the expression of *AthMA4* ([Xu et al., 2010](#)). Therefore, the addition of salinity probably strengthened the plant's defensive ability against oxidative stress by increasing the activities of antioxidative enzymes ([Fig. 2](#)) or changing the root traits ([Fig. 1](#)) and even affecting the expression of heavy metal transporters ([Xu et al., 2010](#)), thereby changing the Cd uptake in the salt-tolerant rice cultivar.

2.4. Cd uptake by rice plants cultured with Cd-polluted saline soil

The soil cultured pot experiment indicated a similar trend of the Cd concentration in the root, shoot, and husk of Chang Xianggu and HH12 ([Table 2](#)). Compared with CK, both the Cd stress and the Cd+Na group significantly increased the Cd

concentration in the root and shoot parts of Chang Xianggu and HH12. The Cd and Cd+Na treatment increased the Cd concentration of rice grain in Chang Xianggu, being 10.68 and 9.03-fold that of CK. Single Cd stress or the combined Cd and Na stress, however, presented no marked difference in the Cd concentration of rice grain in HH12. The Cd concentration of rice grain in Chang Xianggu was much higher than those of HH12 under both the Cd treatment and the Cd+Na group, being 1.94 and 2.39 times, respectively. These results implied a higher food security risk for Chang Xianggu planted in saline soil with Cd contamination, but the Cd concentrations in rice grain of HH12 and Chang Xianggu were both lower than the food safety standard of China (NY861-2004, ≤ 0.2 mg/kg). This soil cultured pot experiment was conducted with a continuous flooding condition during rice growth, which could easily lead to lower Cd content of rice root, shoot, and grain at the mature stage ([Ye et al., 2018](#)). This may be one of the reasons why Cd uptake in these two rice plants was relatively low in this work. Furthermore, these results denoted that a flooding condition probably weakened the salinity effect on Cd accumulation in sea rice ([Table 2](#)).

TF (Transfer Factor) is an important index to evaluate the ability of plants to extract pollutants ([Tang et al., 2020](#)). However, the $TF_{shoot/root}$ of HH12 was both higher than 1.0 under single Cd stress and Cd+Na group, while the $TF_{grain/husk}$ of HH12 with combined Cd and saline stress was lower than 1.0. These results indicated a high potential of transporting Cd from root to shoot in HH12, but with a low risk of transferring Cd to rice grain, which could be a potential feature to keep lower Cd toxic risk in rice grain. Chang Xianggu, however, presented a higher $TF_{grain/husk}$, being 2.26 and 4.49-folds that of HH12 under the Cd and Cd+Na treatment ([Table 2](#)). The shoot Cd accumulation of HH12 was higher both with the treatment of Cd addition or the combined Cd and Na treats, compared with CK (4.72 and 4.90 folds, respectively). Chang Xianggu is reported to be a suitable plant for the phytoremediation of paddy soil with moderately low Cd contamination ([Ishikawa et al., 2006; Murakami et al., 2009](#)). Our results indicated that the Cd uptake by HH12 (64.45 $\mu\text{g}/\text{plant}$ in the

Table 2 – Cd concentration, TF, and BCA of HH12 and Chang Xianggu under different salinity and Cd stress.

Items		Chang Xianggu						HH12					
		CK		Cd		Cd/Na		CK		Cd		Cd/Na	
Cd concentration (mg/kg)	Root	0.22	± 0.06 Bb	1.3	± 0.22 Aa	1.25	± 0.23 Aa	0.36	± 0.04 Bb	1.4	± 0.10 Aa	1.34	± 0.16 Aa
	Shoot	0.21	± 0.04 Bb	0.76	± 0.15 Ba	0.83	± 0.21 Ba	0.19	± 0.03 Bb	1.97	± 0.56 Aa	1.87	± 0.06 Aa
	Husk	0.03	± 0.02 Ba	0.07	± 0.01 ABa	0.05	± 0.00 ABa	0.08	± 0.02 ABa	0.07	± 0.01 ABa	0.12	± 0.04a A
	Brown rice	0.02	± 0.02 Cb	0.17	± 0.04 Aa	0.15	± 0.05 ABa	0.08	± 0.02 ABCa	0.09	± 0.03 ABCa	0.06	± 0.01 BCa
TF	TF	0.97	± 0.14 ABa	0.60	± 0.08 Ba	0.65	± 0.08 Ba	0.52	± 0.03 Aa	1.46	± ± 0.45 Aa	1.44	± 0.21 Aa
	shoot/root	0.19	± 0.10 Ba	0.10	± 0.03 Ba	0.07	± 0.02 Ba	0.42	± ± 0.05 Aa	0.05	± 0.02 Bb	0.06	± 0.02 Bb
	husk/shoot	0.00	± 0.00 Cb	2.58	± 0.69 ABa	3.01	± 0.93 Aa	0.92	± 0.05 Ca	1.14	± 0.13 BCa	0.67	± 0.22 Ca
	grain/husk	0.00	± 0.00 Cb	2.58	± 0.69 ABa	3.01	± 0.93 Aa	0.92	± 0.05 Ca	1.14	± 0.13 BCa	0.67	± 0.22 Ca
Total Cd accumulation	Root	1.08	± 0.06 Cb	12.01	± 2.72 Aa	9.86	± 2.01 ABa	4.92	± 0.45 BCa	7.68	± 2.00 ABa	6.22	± 1.17 BCa
	Shoot	17.43	± 3.74Bb	38.89	± 9.77 ABab	55.26	± 15.28 Aa	13.15	± 1.86 Bb	62.07	± 14.98 Aa	64.45	± 6.54 Aa

TF= transfer factor. Lowercase letters on each line indicate the significant differences between different treatments of Chang Xianggu and HH12, respectively ($p < 0.05$). The capital letter on each line indicates significant differences between Chang Xianggu and HH12 with different treatments ($p < 0.05$). Data were presented as mean ± standard error ($n = 3$).

shoot) was close to that of Chang Xianggu (55.26 $\mu\text{g}/\text{plant}$) under the co-stress of Cd and salinity at the same continuous flooding condition, suggesting that sea rice might have a certain Cd phytoremediation potential under Cd-polluted saline soil. But additional work was required to verify the phytoremediation ability involved in Cd removal in sea rice exposed to co-stress of Cd and salinity in an actual field condition.

Generally, the NaCl addition increases the Cd mobility in the soil thus enhancing the Cd concentration or accumulation in plant tissues (Lutts et al., 2016). However, our work indicated that add-NaCl showed limited influence on the Cd concentration of rice tissues in the soil-cultured system. On the one hand, researchers report that NaCl paradoxically suppressed the heavy metal-induced growth inhibition (Filipović et al., 2018; Zhou et al., 2019). For example, some researchers prove that salinity reduced Cd accumulation in Mediterranean halophyte species (Lefèvre et al., 2009), or the cotton species (Ibrahim et al., 2015). Others argued that NaCl induces higher Cd solubility in the soil thereby increasing the risk of rice uptaking more Cd, and the salinity-induced osmoregulation can greatly trigger the biogeochemical processes involved in Cd uptake by plants (Xu et al., 2017). On the other hand, soil salt stress is a complex environment, with both water stress components associated with a low osmolality of soil solution and ion stress components related to the accumulation of toxic ions (Lutts and Lefèvre, 2015; Stevens et al., 2004). Though with an increase in heavy metal mobility in soil, the mobilized heavy metals may not be absorbed by the plant roots (Cheng et al., 2012; Wahla and Kirkham, 2008). The final Cd uptake by plants may be influenced by the salinity intensity, water condition, plant species, etc.

2.5. Na concentration in rice plant with different Cd and saline levels

The different Na, Cd treatments and the Cd \times Na interaction presented different tendencies on the Na concentration in HH12 and Chang Xianggu (Fig. 5). Without salinity stress, Cd treatments showed no effect on Na concentration in HH12. But at high Cd level, shoot Na concentration of HH12 notably increased, increasing by 66.53% and 55.78%, respectively at low Na and high Na levels (Fig. 5a). The shoot Na concentration of Chang Xianggu denoted no marked differences among Na2Cd0, Na2Cd1, and Na2Cd2 treatments (Fig. 5b). Similarly, based on the ANOVA analysis, the Na treatment ($p < 0.01$), Cd treatment ($p < 0.01$), and the Cd \times Na interaction ($p < 0.01$) all showed a considerable effect on the shoot Na concentration of HH12, but only Na treatment markedly affected shoot Cd concentration of Chang Xianggu ($p < 0.01$).

At a low Na level, Na1Cd2 considerably enhanced Na concentration in HH12 root, compared with Na1Cd0 (Fig. 5c). At high Na levels, Na2Cd1 and Na2Cd2 also increased Na concentration in HH12 root, increasing by 58.25% and 49.14%, respectively, compared with Na2Cd0. As for Chang Xianggu, at low Na level or high Na level, low Cd treatment (Na1Cd1 and Na2Cd1) all decreased root Na concentration, decreasing by 71.00% and 44.57%, compared with Na1Cd2 and

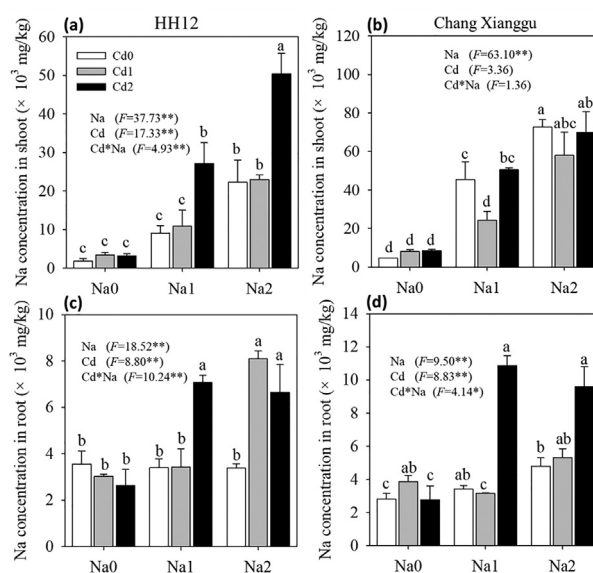


Fig. 5 – Na concentration in the shoot and root of HH12 (a, c) and Chang Xianggu (b, d) under different salinity and Cd stress. Lowercase letters on the bar chart indicate significant differences among different treatments ($p < 0.05$). And **denotes $p < 0.01$, *denotes $p < 0.05$, the same below. And data were presented as mean \pm standard error ($n = 3$).

Na2Cd2, respectively (Fig. 5d). Based on the ANOVA analysis, the Na treatment ($p < 0.01$), Cd treatment ($p < 0.01$), and the Cd \times Na interaction ($p < 0.01$) all notably influenced root Na concentration in HH12 and Chang Xianggu ($p < 0.01$).

There was a clear demarcation between the two rice genotypes in their tolerance and sensitivity. Chang Xianggu was more sensitive to stresses, as characterized by high Cd and Na concentration. Additionally, it was interesting to find that the Cd supplement also notably affected the Na concentration in both the shoot and root of HH12 (Fig. 5). This result is similar to Ibrahim et al. (2015), which denotes that the combined Cd and saline stress causes a notable increase in Na concentration in cotton species (Ibrahim et al., 2015). But Mühling and Läuchli (2003) prove that the combined Cd and Na stress significantly decreases Na concentration in both salt-sensitive and salt-tolerant wheat genotypes. As discussed above, the interaction between Cd and salinity would probably be due to the increase of plant defensive ability against oxidative stress, and even the expression of heavy metal transporters. But the interaction between Cd and NaCl in soil was far more complicated and is affected by many other factors, such as the plant species and growth conditions (Ibrahim et al., 2015). However, it is noteworthy to illustrate the interaction between Cd and salt stress in the salt-tolerant species to evaluate the effect of NaCl on Cd absorption independently of Cd bioavailability in a hydroponic condition (Lutts and Lefèvre, 2015).

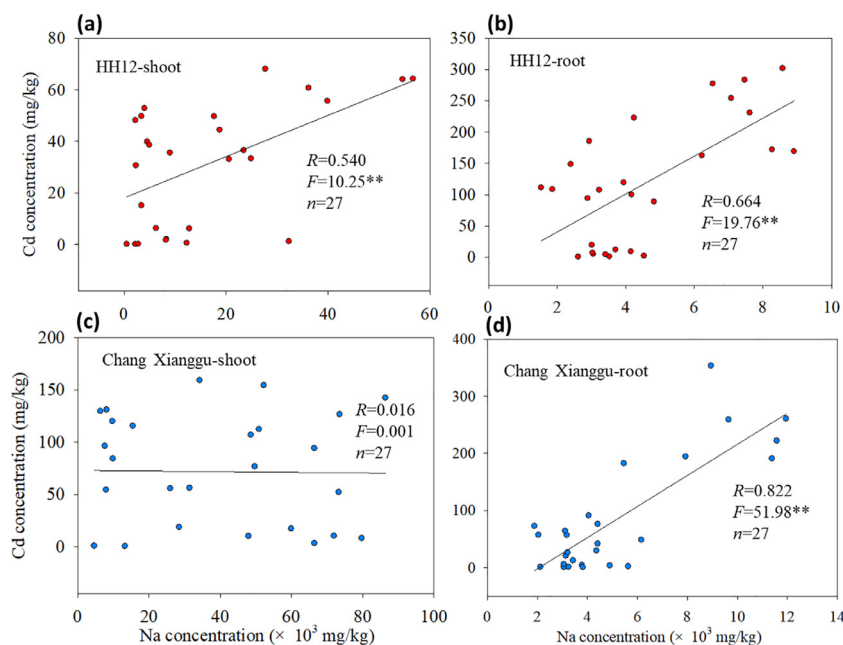


Fig. 6 – Correlation analysis between Cd and Na concentrations in the shoot (a, c) and root (b, d) of HH12 and Chang Xianggu under different salinity and Cd stress. ** denotes $p < 0.01$, * denotes $p < 0.05$.

2.6. Correlation analysis between Cd and Na concentrations

According to the correlation analysis, there was a significant positive correlation between the Cd concentration and the Na concentration in the shoot and root of HH12 ($p < 0.01$) (Fig. 6a, b). Cd level rose considerably with a concomitant increase in Na level in both shoot and root of HH12, and vice versa. Conversely, shoot Cd concentration in Chang Xianggu showed no significant correlation with Na concentration ($p > 0.05$) (Fig. 6c). But root Cd concentration, however, presented a significant positive correlation with the Na concentration in Chang Xianggu ($p < 0.01$) (Fig. 6d). It denoted that the responses of root and shoot to the combined Cd and saline stress were different, and the root response seemed to be more complicated. Additionally, it suggested that Cd and Na showed a good synergistic effect in HH12.

3. Conclusion

It was revealed from the hydroponic experiment that Cd level rose significantly with a concomitant increase in Na level in both shoot and root of sea rice, but not the high Cd-accumulating rice cultivar. i.e., Cd level rose significantly with a concomitant increase in Na level and vice versa. Lower MDA content was found in HH12, indicating an increase in the defensive ability against oxidative stress with Cd and salinity stress. The soil-cultured pot experiment showed that the co-stress of Cd and salinity further inhibited the rice growth and rice yield of sea rice. High $TF_{shoot/root}$ of HH12 indicated a potential of transporting Cd from root to shoot in HH12, but with a low risk of transferring Cd to rice grain, and the Cd con-

centration of rice grain met the national food safety limits. Furthermore, the Cd extracts by sea rice were close to the reported high-Cd- accumulating Chang Xianggu. Additional work is however required to precisely identify the phytoremediation ability involved in Cd removal in sea rice exposed to salinity in the presence of Cd.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this article.

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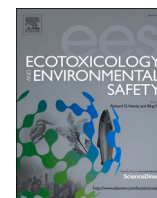
Appendix A Supplementary data

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.jes.2022.05.053.

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Role of passivators for Cd alleviation in rice-water spinach intercropping system

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ABSTRACT

Soil pollution with cadmium (Cd) has posed a threat to our food safety. And rice consumption is the main source of Cd intake in China. Rice intercropping with water spinach is an efficient way for crop production and phytoremediation in Cd-contaminated soil. However, few people work on the Cd remediation by a combination of the passivation and intercropping. In this study, two passivators (the Si-Ca-Mg ameliorant and the Fe-modified biochar with microbial inoculants) were used in the monoculture and intercropping systems to evaluate the potential of co-effect of passivators and cropping systems on the plant growth and Cd phytoremediation. Results showed that the highest rice biomass and rice yield were presented in the intercropping system with the passivator additions, however, relatively lower biomass was showed in water spinach due to the competition with rice. And more Cd accumulated in water spinach while lower Cd in that of different rice parts. The intercropping system with the addition of the Si-Ca-Mg ameliorant and the microbial Fe-modified biochar significantly reduced the Cd contents in brown rice by 58.86% and 63.83%, while notably enhanced the Cd accumulation of water spinach by 32.0% and 22.0%, compared with the monoculture without passivation, respectively. This probably due to the increased pH, the lowered Cd availability in soil, and the reduced TF and BCF values in rice plants with passivator applications. Collectively, this study indicated that rice-water spinach intercropping, especially with the passivator additions, may function as an effective way for Cd remediation and guarantee rice grain safety.

1. Introduction

Soil serves vital functions of food production, and healthy soil is a crucial way to ensure our food safety (Hu et al., 2013). However, heavy metal pollution of soils has remarkably increased due to the unconscionable anthropogenic activities in recent decades (Ghosh and Singh, 2005). And Cd is one of the widespread harmful heavy metals in the environment that possesses high mobility and toxicity to living organisms (Song et al., 2015). Based on the National Investigation Bulletin of Soil Pollution Status (NIBSPS) issued by the Ministry of Environmental Protection of China (MEP-PRC), it denotes that the anthropogenic activities such as industry, mining and agriculture are the main causes of soil pollution in China (including the disposal of industrial effluents and mining wastes, and the agricultural application of sewage sludge). The

prominent heavy metal-contaminated regions are presented in the Yangtze River Delta, the Pearl River Delta, and the old industrial bases in Northeast China (Fan et al., 2016; Sun et al., 2019). And the land area impaired by Cd contamination in China is over about 7%, and the Cd pollution incidents mainly occurred in the southern part of China (Wu et al., 2018). Therefore, high Cd accumulation in paddy rice (main crop in South China) potentially leads to serious impacts on public health (Luo et al., 2017). For example, the case of "Itai-Itai disease" happened in Japan during the 1950s was resulted from the long-term intake of Cd-polluted rice (Huang et al., 2009; Yamagata and Shigematsu, 1970). Therefore, it is indispensable to take action to remediate the Cd-contaminated paddy soils.

There are three major measures used to lower the bioavailability of Cd currently, i.e. isolation, removal, and stabilization (Ji et al., 2011). It

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is well documented that passivators can change the tolerance of plants to Cd toxicity and affect Cd accumulation in different plant parts (Stoddard et al., 2017). By present, in-situ passivation technology has been widely used in soil remediation in China (Zhang et al., 2019). Biochar, for example, a carbon-rich material produced under anaerobic or oxygen-limited condition, has been universally applied in soil remediation (Godlewski et al., 2017). Numerous published papers have documented the ability of biochar to adsorb heavy metals and to reduce the mobility, bioavailability, and consequently toxicity of heavy metals in the soil (Wei et al., 2019). Notably, the mixture of biochar with functional bacterial or fungal strains is another effective strategy for sustainable remediation recently (Chen et al., 2019; Tu et al., 2020). Moreover, the silicon-rich materials are another effective method to repair Cd-polluted soil in China. Various studies have demonstrated that the addition of silicon (Si) in the soil can improve plant growth and reduce Cd accumulation in edible plant parts (Cai et al., 2020; Rehman et al., 2019).

Compared with physical and chemical techniques, phytoremediation is a cost-effective and environmentally friendly technology for remediation (Ji et al., 2011). Previous research showed that intercropping can increase the biomass yield and metal accumulation ability of some hyperaccumulators, while decreased the metal accumulation in the target crop plants (Luo et al., 2019; Xia et al., 2018). But rice plants are hydrophyte, it is rare to intercrop with xeric hyperaccumulators. However, water spinach is reported to accumulate relatively high levels of Cd from the soil (Hu et al., 2013), and intercropped well with paddy rice in our previous study (Kang et al., 2020). Moreover, Ning et al. (2017) suggested that the rice/water spinach intercropping show a yield advantage for rice plants, improve Si nutrition, and control disease and pest for paddy rice (Ning et al., 2017). Therefore, the intercropping of paddy rice and water spinach might be a practical way to remediate the Cd-contaminated soil without impeding agricultural production.

Based our previous field experiment, we compared the effects of four passivators on rice yield and the Cd concentrations in different parts of rice (Table S1). And the Si-Ca-Mg ameliorant and the Fe-modified biochar with microbial inoculants presented the best overall effects in this field experiment (higher rice yield with reduced Cd concentration in brown rice). Therefore, in this study, the Si-Ca-Mg ameliorant and the Fe-modified biochar with microbial inoculants were further used in the monoculture and intercropped culture of the rice plant and water spinach, to evaluate the Cd phytoremediation by the co-work of passivation and intercropping.

2. Materials and methods

2.1. Soil preparation

The rice variety used in the pot experiment was Huang Huazhan. This rice variety is widely grown in Guangdong Province, South China and provided by Rice Research Institute in Guangdong Academy of Agricultural Sciences. And typical water spinach (*Ipomoea Aquatica* Forsk) cultivar (common variety in the market) was used in this work, attained from the Guangxi Zilong Seed co., LTD. The topsoil (0–30 cm) in the experimental field of the Ecology Department, South China Agricultural University (23°16'N, 113°37'E) was collected and homogenized as the tested soil. The collected soil sample was dried naturally and sifted by 2 mm sieve, without any soil gravel, leaves, and other debris. The pH of the soil was 5.81, the organic matter content was 25.02 g/kg. The total N content in soil was 1.87 g/kg, the available P content was 41.9 mg/kg, and the total K content was 46.20 mg/kg. The final total Cd content in soil was 0.96 mg/kg and was supplied as CdCl₂·5/2H₂O. The tested soil was thoroughly blended with the CdCl₂·5/2H₂O for three months to stay homogeneous.

2.2. Experimental design

After germination, the uniform and healthy rice plants (25-day cultivation) and water spinach seedlings (10-day cultivation) were transplanted to the plastic pot (50 kg soil, length × width × height = 50 × 40 × 28 cm). There were nine treatments in this study: (1) rice monoculture (R) + without passivator (T0), Si-Ca-Mg ameliorant (T1), Fe-modified biochar with microbial inoculants (T2); (2) water spinach monoculture (S) + without passivator (T0), Si-Ca-Mg ameliorant (T1), Fe-modified biochar with microbial inoculants (T2); and (3) rice-water spinach intercropping (I) + without passivator (T0), Si-Ca-Mg ameliorant (T1), Fe-modified biochar with microbial inoculants (T2). Different treatments were organized with three replications in a randomized factorial design. The addition levels of Si-Ca-Mg ameliorant, Fe-modified biochar, and microbial inoculants were 30.0, 30.0, and 1.0 g per pot respectively. Six rice seedlings were planted per pot in the rice monoculture system, with a row spacing of 24 cm and 17 cm respectively; Twenty-four water spinach seedlings were planted per pot in the water spinach monoculture system, with a row spacing of 8 cm respectively; as for the intercropping of rice and water spinach, four rice seedlings and eight water spinach seedlings were planted in each pot, with a row spacing of 12 cm. The main component features of these two passivators were shown in Table S2. And the tabulated form of each treatment was listed in Table S3. The powder microbial inoculants were provided by Shenzhen Spurui Biotechnology Co., Ltd., with a mixture of *Bacillus subtilis*, microzyme, lactic acid bacteria, and flocculent bacteria, etc.; and the total number of live bacteria in this microbial inoculants was $\geq 5.0 \times 10^9$ cfu/g. The water surface was 1–2 cm higher than the soil surface during the growth process, and a compound fertilizer (15-15-15) was used to maintain N, P, K balance for rice and water spinach growth. Rice and water spinach were harvested at the heading and maturity stages, and only the above-ground parts of the water spinach were harvested at the heading stage.

2.3. Sampling and analysis

2.3.1. Analysis of soil property and plant growth

Before starting the pot experiment, the pH, the organic matter content, and the N, P, K contents in soil were measured. The soil pH was measured by a pH glass electrode at the water: the solid ratio of 2.5:1. The soil carbon content was determined by the potassium dichromate oxidation method. The total N was determined by the semi-micro-Kjeldahl method after the soil was digested by HF-HClO₄, the available P was determined by acidic molybdate-ascorbic acid blue color method and the total K was determined by the flame photometry method (Bao, 2008; Yang et al., 2019b).

Plants were harvested respectively at the heading and maturity stage. Different samples of the rice plant (grain, straw, root, and soil) and water spinach (shoot, root, and soil) were separated and collected, and the roots were rinsed successively with tap water and distilled water to remove the attached impurities (Yang et al., 2019b). The fresh biomass of rice and water spinach were obtained, then oven-dried at 70 °C to attain a constant weight. The thousand seed weight and the average yield per rice plant were also calculated at the ripening stage.

2.3.2. Determination of Cd content in soil and plant parts

The dried plant samples were ground in a stainless steel mill, and digested by microwave digestion (MARS6, CEM Corporation, USA) with the mixed HNO₃-H₂O₂ solution. At the same time, the quality control (CDHK-GBW(E)100349, Certified reference material for the chemical composition of rice flour) and the blank samples were generated. The Cd form fractions of soil samples were extracted by the BCR method, i.e. the acid-exchangeable fraction (F1), the reducible fraction (F2), the oxidizable fraction (F3) and the residual fraction (F4) (Lei et al., 2020). The Cd content of soil and plant samples were then detected by graphite furnace atomic absorption spectrometry (Z700P, Jena, Germany) (Yang

et al., 2019a). The Cd recovery rate of the reference material was $86.99\% \pm 4.53$. The reagents used in the experiments were of AR.

2.4. Data analysis

Experimental data was performed using a one-way or two-way analysis of variance (ANOVA) and the means compared using a significant difference (Duncan) method at a 5% level (SPSS 17.0). All data are presented as mean \pm standard deviation ($n = 3$). And figures were finished using Origin 8.5.

The calculation of the bioconcentration factor (BCF) was as followed: $BCF = C_p/C_s$, where C_p and C_s refer to the Cd contents in the plant and soil, respectively, in mg/kg.

The calculation of transfer factors (TF) was as followed:

$TF = C_{\text{tissue1}}/C_{\text{tissue2}}$, where C_{tissue1} and C_{tissue2} refer to the Cd contents in the different parts of the rice plant, in mg/kg.

The calculation of Cd extraction per hectare was as followed:

Cd extraction amount = Cd accumulation per plant (rice or water spinach) multiplied by the number of plants per hectare (Zeng et al., 2019).

3. Results

3.1. Plant biomass

The addition of passivators promoted the rice growth and rice yield in the rice-water spinach intercropping system (Table 1). The roots, stems, leaves, yield, and the 1000-grain weight of rice plants all showed a trend of $T1 > T2 > T0$ under the same cropping system. The highest biomass of root, stem, and leaves of rice plants was showed in IT1 treatment at the heading stage. Based on the ANOVA analysis, both the cropping system ($F = 21.73$, $p < 0.01$) and passivators ($F = 9.21$, $p < 0.01$) showed a significant effect on rice biomass. At the maturity stage, T1 and T2 treatments remarkably increased the root biomass of monoculture rice, increasing by 22.85% and 26.13% respectively compared with T0 treatment. And T1 and T2 treatments notably increased the stem and leaf biomass, and the rice yield of rice-water spinach intercropping system ($p < 0.05$). The ANOVA analysis showed that both the cropping system ($F = 14.88$, $p < 0.01$) and passivators ($F = 6.91$, $p < 0.01$) had a notable effect on rice biomass. But only cropping system significantly affected the 1000-grain weight ($F = 47.44$, $p < 0.01$) and the rice yield ($F = 14.07$, $p < 0.01$).

Conversely, the addition of passivators significantly reduced the shoot biomass of water spinach in the rice-water spinach intercropping system at the heading stage, decreasing by 33.33% (T1) and 38.46% (T2) when compared with T0. Based on the ANOVA analysis, the cropping system ($F = 11.11$, $p < 0.01$), the passivators ($F = 7.51$, $p < 0.01$), and the interaction of cropping system and passivator ($F = 4.08$, $p < 0.05$) all showed a notable effect on the shoot biomass of water spinach.

At the maturity stage, T1 and T2 treatments decreased the root and shoot biomass of water spinach under the monoculture system ($p < 0.05$). And highest water spinach biomass was found in T0 treatment. And the ANOVA analysis indicated that both the passivators ($F = 12.73$, $p < 0.01$), and the interaction of the cropping system and passivator ($F = 4.61$, $p < 0.05$) notably affected the water spinach biomass.

3.2. Cd-accumulating features

3.2.1. Cd-accumulating features of rice

The addition of passivators reduced the Cd concentration and BCF of rice plants in the rice-water spinach intercropping system (Table 2). At the heading stage, T1 treatment significantly decreased the Cd concentrations of rice root, stem, leaves, and the bioconcentration factor of the root in the intercropping system, compared with RT0 treatment. And T2 treatment lowered Cd concentration in rice stem (57.6%) and the transfer factor of the root to stem (53.1%). Based on the ANOVA analysis, the passivator treatments significantly influenced the Cd concentration of rice root and stem, and the BCF_R ($p < 0.01$). At the maturity stage, lowest Cd concentrations of rice root, stem, leaves, and the BCF_R were found in the rice-water spinach intercropping system ($p < 0.05$) under T1 treatment. And IT1 treatment notably reduced the Cd concentration in brown rice, decreasing by 58.9% and 49% respectively, compared with RT0 and IT0. Comparably to RT0, RT2 treatment showed no obvious effect on the Cd concentrations, TFs, and BCF_R , while IT2 treatment markedly decreased the Cd concentrations of rice root, leaves, and brown rice ($p < 0.05$). But the passivator additions indicated no significant effects on the transfer factors under different treatments ($p > 0.05$). Based on the ANOVA analysis, both the cropping system ($p < 0.01$) and the passivators ($p < 0.01$) showed a notable effect on the Cd concentrations of rice roots, leaves, brown rice, and the BCF_R .

3.2.2. Cd-accumulating features of water spinach

The addition of passivators increased the Cd concentration and BCFs of water spinach in the intercropping system (Table 3). At the heading stage, under the same passivating agent treatment, the shoot Cd content of water spinach showed a trend of monocropping system < intercropping system; and the trend of the shoot Cd concentration of water spinach in the monocropping system presented the tendency of $T1 > T2 > T0$. And T1 and T2 treatments notably increased the shoot Cd concentration of water spinach in the intercropping system, increasing by 37.5% and 50.98% respectively compared with T0. Based on the ANOVA analysis, the cropping system ($p < 0.01$), the passivators ($p < 0.01$), and the interaction of the cropping system and passivator ($p < 0.01$) all showed a notable effect on the shoot Cd concentration and the BCF_{shoot} of water spinach.

The trend of the Cd-accumulating features of water spinach at the

Table 1
Plant biomass of different treatments (g/plant).

Treatments	Root	Stem	Leaves	1000-grain weight	Rice yield	Root	Shoot
Heading stage	RT0	2.29 \pm 0.17c	25.10 \pm 1.897 b	14.05 \pm 0.960 b	/	ST0	48.70 \pm 3.66a
	RT1	3.15 \pm 0.15 ab	27.71 \pm 2.505 ab	16.31 \pm 1.741 ab	/	ST1	42.95 \pm 4.06a
	RT2	3.05 \pm 0.14 b	25.74 \pm 1.728 b	16.21 \pm 2.612 ab	/	ST2	47.52 \pm 1.69a
	IT0	3.36 \pm 0.13 ab	28.17 \pm 1.865 ab	15.96 \pm 1.195 ab	/	IT0	49.81 \pm 4.03a
	IT1	3.51 \pm 0.12a	33.17 \pm 1.894a	19.35 \pm 1.029a	/	IT1	32.47 \pm 2.01 b
	IT2	3.34 \pm 0.09 ab	28.52 \pm 1.893 ab	17.40 \pm 1.027 ab	/	IT2	29.97 \pm 3.49 b
Maturity stage	RT0	2.87 \pm 0.11 b	27.53 \pm 1.253 b	14.75 \pm 0.356c	16.27 \pm 0.222c	ST0	19.62 \pm 2.295a
	RT1	3.72 \pm 0.28a	29.32 \pm 2.033 b	16.79 \pm 0.978bc	16.65 \pm 0.297c	ST1	10.02 \pm 0.802c
	RT2	3.62 \pm 0.10a	28.54 \pm 1.569 b	17.32 \pm 1.573 abc	16.67 \pm 0.212c	ST2	14.36 \pm 1.321bc
	IT0	4.00 \pm 0.12a	29.48 \pm 1.674 b	16.75 \pm 0.668bc	18.57 \pm 0.218a	IT0	18.00 \pm 2.183 ab
	IT1	4.15 \pm 0.19a	35.49 \pm 2.358a	20.35 \pm 0.511a	17.55 \pm 0.318 b	IT1	16.34 \pm 1.131 ab
	IT2	4.05 \pm 0.17a	30.18 \pm 1.481 ab	18.37 \pm 1.343 ab	17.47 \pm 0.074 b	IT2	15.32 \pm 0.770 ab

T0 = without passivator; T1 = Si-Ca-Mg ameliorant; T2 = Fe-modified biochar with microbial inoculants; R = rice; S = water spinach; I = rice and water spinach intercropping system. Lowercase letters on each row indicate significant differences among different treatments ($p < 0.05$). All values are presented as mean \pm standard error ($n = 3$).

Table 2

Cd-accumulating features of rice under different treatments.

Treatments		Cd concentration (mg/kg)				TF _{S/R}	TF _{L/S}	TF _{G/L}	BCF _R
		Root	Stem	Leaves	Brown rice				
Heading stage	RT0	1.04 ± 0.04a	0.33 ± 0.033a	0.13 ± 0.014a	/	0.32 ± 0.045 ab	0.42 ± 0.08 ab	/	1.08 ± 0.04a
	RT1	0.88 ± 0.06 ab	0.31 ± 0.023a	0.10 ± 0.007 ab	/	0.36 ± 0.005a	0.33 ± 0.01 b	/	0.91 ± 0.07 ab
	RT2	0.99 ± 0.04a	0.22 ± 0.024bc	0.12 ± 0.012 ab	/	0.22 ± 0.032bc	0.56 ± 0.13 ab	/	1.03 ± 0.04a
	IT0	1.00 ± 0.06a	0.28 ± 0.031 ab	0.10 ± 0.008 ab	/	0.28 ± 0.035 ab	0.35 ± 0.06 ab	/	1.04 ± 0.07a
	IT1	0.81 ± 0.03 b	0.21 ± 0.029bc	0.08 ± 0.007 b	/	0.26 ± 0.030 ab	0.39 ± 0.02 ab	/	0.85 ± 0.03 b
	IT2	0.98 ± 0.04a	0.14 ± 0.013c	0.09 ± 0.016 b	/	0.15 ± 0.019c	0.64 ± 0.14a	/	1.02 ± 0.04a
Maturity stage	RT0	2.21 ± 0.17a	0.26 ± 0.017a	0.18 ± 0.006a	0.141 ± 0.010a	0.12 ± 0.017a	0.71 ± 0.060a	0.76 ± 0.043a	2.30 ± 0.18a
	RT1	1.57 ± 0.10 b	0.20 ± 0.014 ab	0.10 ± 0.014c	0.070 ± 0.013cd	0.13 ± 0.007a	0.50 ± 0.107a	0.78 ± 0.217a	1.64 ± 0.11 b
	RT2	2.08 ± 0.06a	0.25 ± 0.023 ab	0.16 ± 0.013 ab	0.110 ± 0.010 ab	0.12 ± 0.011a	0.67 ± 0.120a	0.69 ± 0.059a	2.16 ± 0.06a
	IT0	1.85 ± 0.16 ab	0.23 ± 0.024 ab	0.14 ± 0.010 b	0.100 ± 0.017bc	0.13 ± 0.012a	0.61 ± 0.078a	0.74 ± 0.163a	1.93 ± 0.17 ab
	IT1	1.13 ± 0.05c	0.18 ± 0.013a	0.08 ± 0.014c	0.058 ± 0.008 d	0.16 ± 0.012a	0.45 ± 0.081a	0.74 ± 0.069a	1.18 ± 0.06c
	IT2	1.53 ± 0.16 b	0.20 ± 0.030 ab	0.09 ± 0.013c	0.051 ± 0.010 d	0.13 ± 0.022a	0.51 ± 0.151a	0.54 ± 0.034a	1.60 ± 0.16 b

TFS/R = transfer factor of the root to stem; TFL/S = transfer factor of the stem to leaves; TFG/L = transfer factor of the leaves to grain; BCF_R = bioconcentration factor of rice root; T0 = without passivator; T1 = Si–Ca–Mg ameliorant; T2 = Fe-modified biochar with microbial inoculants; R = rice; I = rice and water spinach intercropping system. Lowercase letters on each row indicate significant differences among different treatments ($p < 0.05$). All values are presented as mean ± standard error ($n = 3$).

Table 3

Cd-accumulating features of water spinach under different treatments (mg/kg).

Treatments	Heading stage		Maturity stage				
	Shoot-Cd	BCF _{shoot}	Root-Cd	Shoot-Cd	TF _{S/R}	BCF _R	BCF _{shoot}
ST0	0.48 ± 0.05c	0.05 ± 0.01 b	1.07 ± 0.17c	0.29 ± 0.02 d	0.29 ± 0.06c	0.11 ± 0.02c	0.03 ± 0.00 d
ST1	0.76 ± 0.10 ab	0.08 ± 0.01 ab	1.13 ± 0.14c	0.86 ± 0.07 b	0.85 ± 0.03a	0.11 ± 0.01c	0.09 ± 0.01 b
ST2	0.53 ± 0.05c	0.04 ± 0.00 b	1.22 ± 0.15bc	0.39 ± 0.08 d	0.34 ± 0.08c	0.13 ± 0.02bc	0.04 ± 0.01 d
IT0	0.50 ± 0.08bc	0.05 ± 0.001 b	1.08 ± 0.13c	0.66 ± 0.04c	0.62 ± 0.04 b	0.11 ± 0.01c	0.07 ± 0.00c
IT1	0.80 ± 0.10a	0.08 ± 0.01a	1.70 ± 0.20 ab	1.32 ± 0.07a	0.80 ± 0.09 ab	0.18 ± 0.02 ab	0.14 ± 0.01a
IT2	1.02 ± 0.12a	0.11 ± 0.01a	1.83 ± 0.19a	0.73 ± 0.03c	0.34 ± 0.03c	0.19 ± 0.02a	0.06 ± 0.00c

TF_{S/R} = transfer factor of shoot to root; BCF_R = bioconcentration factor of water spinach root; BCF_{shoot} = bioconcentration factor of water spinach shoot. T1 = Si–Ca–Mg ameliorant; T2 = Fe-modified biochar with microbial inoculants; S = water spinach; I = rice and water spinach intercropping system. Lowercase letters on each row indicate significant differences among different treatments ($p < 0.05$). All values are presented as mean ± standard error ($n = 3$).

maturity stage was similar to those at the heading stage, and the highest Cd concentrations in the shoot were found in IT1 treatment. And T1 treatment remarkably increased the BCF_R and BCF_{shoot} in both monoculture and intercropping systems, comparing with T0 treatment ($p < 0.05$). Based on the ANOVA analysis, both the cropping system ($p < 0.01$) and the passivators ($p < 0.01$) showed a notable effect on the shoot Cd concentrations and BCF_{shoot} of water spinach, but only the cropping system ($p < 0.01$) significantly influenced the root Cd concentrations and BCF_R of water spinach.

3.2.3. Cd extractions in plants

The Cd accumulation of rice plants was expressed as a tendency of monoculture > intercropping (Fig. 1a). In monoculture, T1 significantly reduced rice Cd accumulation when compared with T0 and T2, decreasing by 21.78% and 23.69% respectively. In the intercropping system, both T1 and T2 notably reduced the Cd accumulation and decreased by 25.08% and 20.63% respectively compared with T0. Similarly, the trend of Cd extraction per hectare of rice was monoculture > intercropping (Fig. 1a). Based on the ANOVA analysis, the passivators ($p < 0.01$) showed a notable effect on the Cd accumulation of rice. Under the monocropping condition, T1 significantly lowered the rice extractions comparing with T0 and T2, which decreased by 21.82% and 23.73% respectively. Additionally, T1 markedly decreased the rice extractions comparing with T0, with a decrease of 25.12%.

The highest Cd accumulation of water spinach was showed in T1 (Fig. 1b). IT1 treatment notably increased the Cd accumulation in water spinach, increasing by 32.0% and 25.2% respectively, compared with ST0 and IT0 treatments. And T-test indicated no significant difference between ST1 and IT1 treatments. Based on the ANOVA analysis, the cropping system ($p < 0.01$) significantly influenced the Cd accumulation of water spinach. Besides, the trend of Cd extraction per hectare of water

spinach was monoculture > intercropping (Fig. 1b). Under the monocropping condition, T1 significantly reduced the Cd extraction of water spinach compared with T2 ($p < 0.05$); but no marked differences were showed in different treatments under intercropping conditions ($p > 0.05$).

3.3. Passivators effect on soil pH

The soil pH significantly increased after the application of T1, but the effect was not significant between the T0 and T2 (Fig. 2). Under the same cropping system, the soil pH showed an inclination of T1 > T2 > T0, where T1 was significantly higher than T0 ($p < 0.05$). Compared with T0, the pH value of RT1, ST1, and IT1 treatments increased by 6.6%, 5.0%, and 4.2%, respectively. At the maturity stage, a notable higher soil pH value was showed in RT1 (pH = 6.18), compared with RT0 (pH = 5.18), while no significant differences presented in water spinach monoculture and the intercropping system. Additionally, T-test indicated that the pH value at the maturity stage was markedly lower than that at the heading stage. Based on the ANOVA analysis, the passivators ($F = 12.35$, $p < 0.01$) significantly influenced the soil pH value at the heading stage.

3.4. Different Cd species in soil

The concentrations of different Cd fractions in soil were in the order of residual fraction > acid-exchangeable fraction > reducible fraction > oxidizable fraction (Fig. 3). The soil Cd fractions significantly transformed after the application of T1. At the heading stage, compared with RT0, the acid-exchangeable fraction of ST1 and IT1 decreased by 60.35% and 72.04%, respectively. And the Cd residual fraction of ST1 increased by 17.01%, comparing with RT1. But at the maturity stage, the

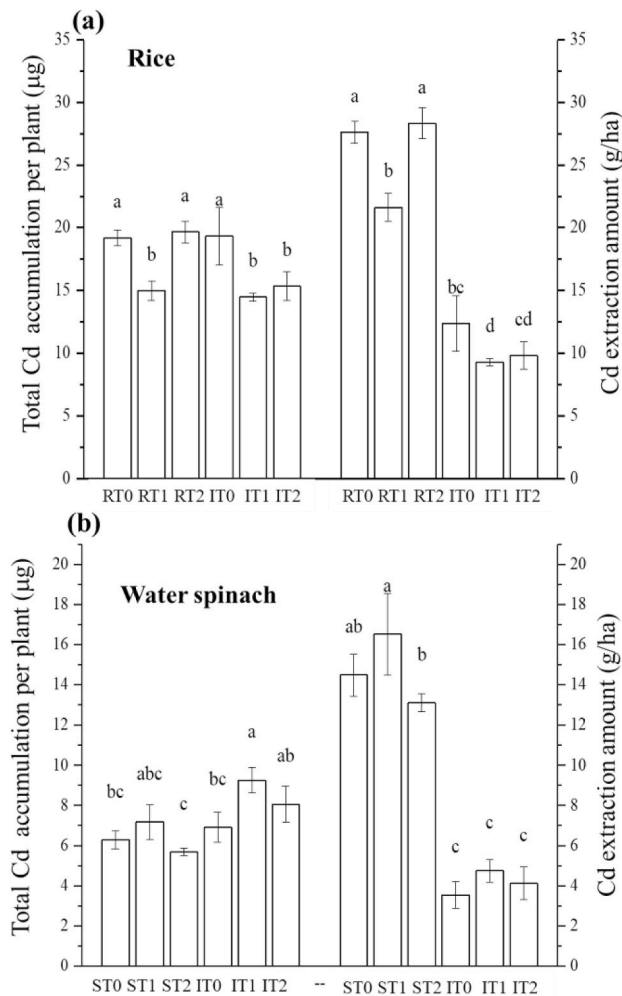


Fig. 1. Cd accumulation and extraction amount of rice (a) and water spinach (b) under different treatments. T0 = without passivator; T1 = Si-Ca-Mg ameliorant; T2 = Fe-modified biochar with microbial inoculants; R = rice; S = water spinach; I = rice and water spinach intercropping system. Lowercase letters on the bar chart indicate significant differences among different treatments ($p < 0.05$). All values are presented as mean \pm standard error ($n = 3$).

passivator additions showed no significant effect on the acid-exchangeable Cd fraction and the reducible Cd fraction ($p > 0.05$). The oxidizable Cd fraction of ST0 was significantly lower than IT0 and IT2, while the residual Cd fractions of ST0 was much higher than that of IT0 (14.28%). Based on the ANOVA analysis, only the passivators ($F = 6.01$, $p < 0.05$) significantly influenced the acid-exchangeable fraction of Cd at the heading stage.

3.5. Correlation analysis of different parameters

The correlation analysis of different parameters of rice plants was presented in Table 4. At the heading stage, a significant negative correlation was found in soil pH and the acid-exchangeable Cd fraction, root Cd concentration, and the BCF ($p < 0.05$). And the rice biomass was negatively correlated with the acid-exchangeable Cd fraction, Cd concentration in root and leaves, and the BCF ($p < 0.05$). While a significant positive correlation was found in the acid-exchangeable Cd fraction and the root Cd concentration and the BCF. Additionally, the contents of the reducible Cd fraction and the oxidizable Cd fraction were negatively correlated with the residual Cd fraction ($p < 0.05$). At the maturity stage, the soil pH was also negatively correlated with the acid-exchangeable Cd fraction. The acid-exchangeable Cd fraction was

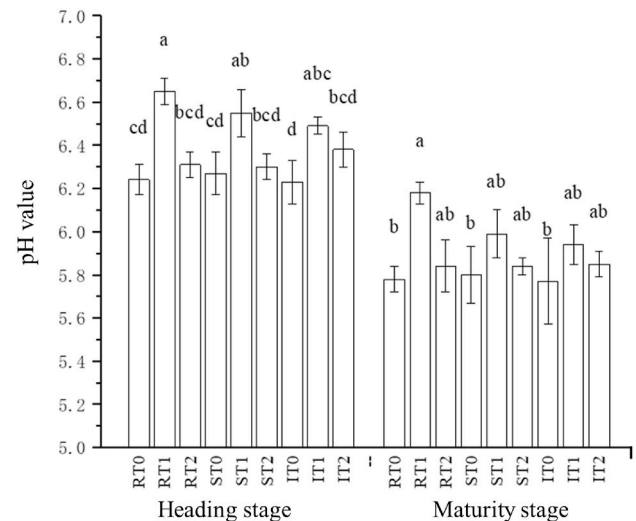


Fig. 2. Soil pH of different treatments at the heading and maturity stages. T0 = without passivator; T1 = Si-Ca-Mg ameliorant; T2 = Fe-modified biochar with microbial inoculants; R = rice; S = water spinach; I = rice and water spinach intercropping system. Lowercase letters on the bar chart indicate significant differences among different treatments ($p < 0.05$). All values are presented as mean \pm standard error ($n = 3$).

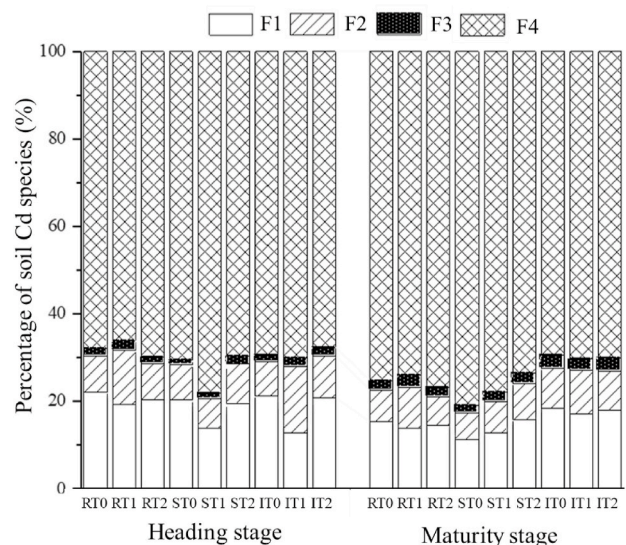


Fig. 3. Cd form fractions of different treatments at the heading and maturity stages. F1 = acid-exchangeable fraction; F2 = reducible fraction; F3 = oxidizable fraction; F4 = residual fraction. T0 = without passivator; T1 = Si-Ca-Mg ameliorant; T2 = Fe-modified biochar with microbial inoculants; R = rice; S = water spinach; I = rice and water spinach intercropping system. All values are presented as mean \pm standard error ($n = 3$).

positively correlated with the reducible Cd fraction and the oxidizable Cd fraction, while it was negatively correlated with the residual Cd fraction ($p < 0.05$). Noticeably, the reducible Cd fraction, rice biomass, and rice yield were negatively correlated with the Cd concentration in brown rice ($p < 0.05$). Meanwhile, the rice yield was also negatively correlated with the BCF, and the Cd concentration in root and leaves ($p < 0.05$).

The correlation analysis of different parameters of water spinach was showed in Table 5. At the heading stage, the soil pH was negatively correlated with the acid-exchangeable Cd fraction and the water spinach biomass, while positively related to the residual Cd fraction ($p < 0.05$). Besides, it indicated that the shoot Cd concentration and the BCF of

Table 4
Pearson correlation of different parameters in soil and rice.

Parameters	pH	F1	F2	F3	F4	Root-Cd	Stem-Cd	Leaves-Cd	Cd accumulation	BCF	Biomass	Rice yield	Brown rice-Cd
Heading stage													
pH	1												
F1	-0.583*	1											
F2	0.157	-0.063	1										
F3	0.253	0.13	0.517*	1									
F4	0.123	-0.448	-0.746**	-0.557*	1								
Root-Cd	-0.548*	0.514*	-0.458	-0.302	0.09	1							
Stem-Cd	0.115	0.178	-0.258	-0.033	0.063	0.074	1						
Leaves-Cd	-0.321	0.436	-0.287	-0.163	-0.017	0.364	0.349	1					
Cd accumulation	0.232	-0.092	-0.172	-0.109	0.088	-0.104	0.898**	0.168	1				
BCF	-0.548*	0.514*	-0.458	-0.302	0.09	1.000**	0.074	0.364	-0.104	1			
Biomass	0.316	-0.620**	0.452	0.086	-0.029	-0.589*	-0.431	-0.747**	-0.068	-0.589*	1		
Maturity stage													
pH	1												
F1	-0.578*	1											
F2	0.115	0.641**	1										
F3	-0.104	0.702**	0.854**	1									
F4	0.298	-0.924**	-0.883**	-0.874**	1								
Root-Cd	-0.393	-0.119	-0.456	-0.334	0.296	1							
Stem-Cd	-0.299	-0.083	-0.446	-0.324	0.27	0.657**	1						
Leaves-Cd	-0.22	-0.249	-0.325	-0.239	0.306	0.727**	0.452	1					
Cd accumulation	-0.433	-0.021	-0.409	-0.228	0.208	0.787**	0.773**	0.584*	1				
BCF	-0.393	-0.119	-0.456	-0.334	0.296	1.000**	0.657**	0.727**	0.787**	1			
Biomass	0.024	0.407	0.475*	0.396	-0.478*	-0.678**	-0.418	-0.700**	-0.27	-0.678**	1		
Rice yield	0.034	0.293	0.333	0.331	-0.345	-0.582*	-0.211	-0.655**	-0.179	-0.582*	0.759**	1	
Brown rice-Cd	-0.28	-0.229	-0.502*	-0.451	0.392	0.797**	0.609**	0.793**	0.797**	0.797**	-0.513*	-0.549*	1

F1 = acid-exchangeable fraction; F2 = reducible fraction; F3 = oxidizable fraction; F4 = residual fraction. n = 18. **p < 0.01, *p < 0.05.

shoot negatively affected the biomass of water spinach. Noticeably, the residual Cd fraction showed a negative correlation with the acid-exchangeable Cd fraction, the reducible Cd fraction, and the oxidizable Cd fraction at the heading and maturity stages ($p < 0.05$). And the water spinach biomass was negatively affected by the shoot Cd concentration, and the BCF of the shoot, and the TF value at the maturity stage ($p < 0.05$). The TF value was positively related to the shoot Cd concentration and the BCF of the shoot ($p < 0.05$).

4. Discussions

4.1. Plant biomass

Intercropping is a conventional cultivation practice that can greatly enhance the use efficiency of farmlands without any additional costs (Lin et al., 2014; Zhao et al., 2019). In this study, our results suggested that both the passivator additions and the cropping systems significantly promoted the rice biomass and the rice yield, while a decrement was showed in the water spinach biomass under the same condition. This probably due to the strong competition for light, water, or nutrients in the intercropping system (Zeng et al., 2019). And the aggressivity (A_{rs}) and the competitive ratio (C_{rs}) in this study indicated that the aggressivity and competitiveness of rice plants were stronger than those of water spinach in the intercropping system (Table S4). Moreover, the LER of T0, T1, and T2 treatments were all higher than 1.0 (Table S4). It indicated better land utilization efficiency in the rice-water spinach intercropping system, compared with sole cropping. These results were consistent with our previous research (Kang et al., 2020), implying that the intercropping of rice and water spinach was a suitable intercropping model, and the passivators addition presented no negative effects on this system.

Notably, the positive effect on plant growth of Si-Ca-Mg ameliorant (T1) was better than the Fe-modified biochar with microbial inoculants (T2), this may be due to the Si-Ca-Mg ameliorant addition better increased the nutrient contents in soil (such as Si), as Si is reported to successfully ameliorate the Cd toxic effect on crops and promote plant growth (Lim et al., 2016). Meanwhile, it indicated that the rice biomass and rice yield was negatively affected by the Cd concentration in rice (Table 4). Therefore, in the intercropping system, the decreased Cd accumulation in rice plants with passivation would alleviate the growth inhibition of Cd toxicity to crops (Rehman et al., 2017).

4.2. pH value and different forms of Cd in soil

Soil pH is one of the most vital parameters to govern Cd speciation, partitioning, and bioavailability (Shahid et al., 2017), and a significant negative correlation was established between soil pH and the exchangeable Cd fraction in this study (Table 4). It is well documented that the additions of Si-rich amendments increase soil pH, and further inhibit the metal uptakes by plants (Sui et al., 2020). It is suggested that a decrease in pH of merely 0.2 units results in a 3–5 times increase in the labile Cd pool (Zhu et al., 2016). When the soil pH increased, the forms of the hydrogen hydroxide will precipitate or chelate compounds, and then lower the effectiveness of heavy metals (González-Núñez et al., 2012). In this work, the soil pH significantly increased after the application of Si-Ca-Mg ameliorant (T1), meanwhile, the soil Cd in acid-exchangeable form notably decreased after the application of Si-Ca-Mg ameliorant (T1) at heading stage. This is consistent with previous findings that Si supplementation successfully alleviates the Cd toxicity on plants (Cai et al., 2020). And we speculated that the increased pH value may be attributed to the liming effect of the alkaline Si-Ca-Mg ameliorant (Table S2). It suggests that the hydrolysis of Si can produce hydroxyl groups in soil resulting in an increment of the soil pH value (Zhao et al., 2020). And the increased soil pH enhances the surface charge on the oxides of Fe, Al, and Mn, thus increasing the organic matter chelating effect and the metal hydroxide precipitation in soil

Table 5
Pearson correlation of different parameters in soil and water spinach.

Parameters	pH	F1	F2	F3	F4	Shoot-Cd	BCFshoot	Biomass					
Heading stage	pH	1											
	F1	−0.610**	1										
	F2	−0.109	0.119	1									
	F3	0.08	0.236	0.493*	1								
	F4	0.529*	−0.656**	−0.774**	−0.540*	1							
	Shoot-Cd	0.312	−0.085	0.198	0.481*	−0.146	1						
	BCFshoot	0.312	−0.085	0.198	0.481*	−0.146	1.000**	1					
	Biomass	−0.553*	0.294	−0.355	−0.465	0.05	−0.700**	−0.700**	1				
Maturity stage	pH	1.000											
	F1	−0.369	1.000										
	F2	−0.077	0.865**	1.000									
	F3	−0.295	0.752**	0.748**	1.000								
	F4	0.281	−0.979**	−0.943**	−0.819**	1.000							
	Root-Cd	0.026	0.387	0.460	0.318	−0.424	1.000						
	Shoot-Cd	0.326	0.268	0.446	0.234	−0.338	0.394	1.000					
	BCFroot	0.026	0.387	0.460	0.318	−0.424	1.000**	0.394	1.000				
	BCFshoot	0.326	0.268	0.446	0.234	−0.338	0.394	1.000**	0.394	1.000			
	TF	0.320	0.073	0.168	0.084	−0.110	−0.224	0.774**	−0.224	0.774**	1.000		
	Biomass	−0.098	−0.242	−0.302	−0.416	0.295	−0.249	−0.475*	−0.249	−0.475*	−0.502*	1.000	

F1 = acid-exchangeable fraction; F2 = reducible fraction; F3 = oxidizable fraction; F4 = residual fraction. n = 18. **p < 0.01, *p < 0.05.

(Bolan et al., 2014).

It is reported that the alkalinity of Fe-based biochar would increase soil pH because the carbonates or oxides base ions that existed in the biochar surface can neutralize the hydrogen ions in soil (Xu et al., 2012). And an increase in pH would be caused by the dissolution of soluble salts in soil (including K and Na carbonates and oxides), after the application of biochar (Joseph et al., 2010). And Sui et al. (2020) proved that biochar aging decreases the formation of acidic functional groups thus promoting the pH in soil (Sui et al., 2020). But in this study, the application of Fe-modified biochar with microbial inoculants presented no significant effect on the pH value and Cd species in soil (Figs. 2 and 3). This result was inconsistent with an earlier report that the Fe-based biochar addition significantly decreased the concentrations of available Cd and As (Tang et al., 2020). This may be related to the application amount of biochar or the difference in the manufacturing process and raw materials of biochar. Tu et al. (2020) proved that the bacteria (*Pseudomonas* sp. NT-2) loaded biochar significantly increase the soil pH, reduce the exchangeable and carbonate bound Cd fractions in the soil, and improve the soil microbial community (Tu et al., 2020). Therefore, it implied the importance to carefully select the biochar types and microbial species.

4.3. Cd accumulation

In this study, the addition of passivators also reduced the Cd concentration of brown rice of rice plants, meanwhile, they notably increased the Cd concentration of water spinach in the intercropping system (Tables 2 and 3). These results were consistent with numerous previous studies on the effects of passivation on heavy metal remediation in the intercropping system (Luo et al., 2019; Zu et al., 2017). In this work, we speculated that the decrease of Cd concentration in rice plants mainly attributed to the Cd competition with neighboring water spinach (Wan et al., 2017), the increased soil pH (Hu et al., 2013) with lower Cd availability (Wang et al., 2020), as evidenced by the negative correlation showed between the soil pH and the acid-exchangeable Cd form (Table 4). Moreover, with passivator addition, the BCF and TF values of rice were also significantly reduced, while those of water spinach increased significantly, compared with monoculture without passivation (Tables 2 and 3). Similar results (decreased Cd concentration in target crops, while increased Cd acquisition in intercropped plants) were also observed in the intercropped pak choi/Sedum (Ma et al., 2020), the intercropped *Pteris vittata*/Morus alba (Wan et al., 2017), and the intercropped *S. nigrum*/eggplant (Tang et al., 2017). And based on our

previous study, the MRER of Cd in the rice-water spinach intercropping system is 1.34, indicating an advantage in removing Cd of this intercropping pattern (Kang et al., 2020). Furthermore, the heavy metal limit standard in the food of China (GB 2762–2012) (Cd in brown rice ≤ 0.2 mg/kg) and the heavy metal limit established by the Codex Alimentarius Commission of FAO/WHO (Cd in brown rice ≤ 0.4 mg/kg) indicated that the Cd concentration of brown rice in the rice/water spinach intercropping system with passivator addition (0.058 mg/kg for T1 treatment, and 0.051 mg/kg for T2 treatment) was far below the food safety standard and was safe for the public (Yang et al., 2019a).

Additionally, it indicated that the addition of Si–Ca–Mg ameliorant (T1) played a significant role in both the monoculture and intercropping systems in the pot experiment, while the supplementation of Fe-based biochar only worked well in the intercropping system. As discussed above, it implied that the additions of passivators increased the soil pH and changed the Cd fractions in soil, thus influenced the Cd accumulation in plants (Hamid et al., 2020). Besides, the application of Si–Ca–Mg ameliorant (T1) increased the soil nutrients (Si in particular), thus increased the plant growth and decreased Cd availability (Adrees et al., 2015). And it is documented that Si may stimulate the production of root exudates to chelate metals (Kidd et al., 2001), and enhanced adsorption of Cd on the cell walls, and even reduce the free metal concentrations in the apoplast or the xylem (Iwasaki et al., 2002; Keller et al., 2015; Ye et al., 2012). Simultaneously, Zeng et al. (2017) report that the Ca addition also decreased Cd uptake in *Arabidopsis thaliana*, by reducing the Cd adsorption in root and decreasing symplastic Cd transport as well as the expression of the genes involved (*AtZIP2* and *AtZIP4*) (Zeng et al., 2017). As for the Fe-modified biochar, although it showed no notable effects on the pH value and Cd morphological change in soil, it significantly increased the Cd concentrations in water spinach and reduced Cd concentration in brown rice (Tables 2 and 3). Due to the large specific surface area of biochar, it plays a better role in improving soil physicochemical and biological properties while effectively absorbing heavy metal ions (Jeffery et al., 2017). Additionally, it is documented that biochar improves the soil microecological environment (Zeng et al., 2019), enhances the soil urease and catalase activities, and promoted the soil microbial community in soil, which leads to better plant growth (Tu et al., 2020).

5. Conclusions

This study provides a new direction into the passivator additions on Cd remediation of rice plant/water spinach under monoculture and

intercropping, respectively. The application of passivators increased soil pH and the Cd residue fractions in the soil. Meanwhile, passivator additions improved the plant biomass, the rice yield, reduced the Cd contents in brown rice, and guaranteed the rice grain safety. The yield-increasing effect of Si–Ca–Mg ameliorant treatment was better than that of Fe-based biochar with microbial inoculants. Simultaneously, rice showed a competitive advantage in the rice and water spinach intercropping system, and the land equivalent ratio was greater than 1.0, indicating that this intercropping system improved the land economic benefits. Overall, the co-work of passivator additions and the rice-water spinach intercropping may be a practicable technology for rice growth and Cd phytoremediation.

Credit author statement

Xu Yang, Conceptualization, Data curation, Formal analysis, Software, Writing - original draft, Writing - review & editing. Wenyan Zhang, Methodology, Data curation, Investigation, Writing - review & editing. Junhao Qin, Conceptualization, Resources, Writing - review & editing. Xuechun Zhang, Methodology, Data curation, Investigation. Huashou Li, Conceptualization, Funding acquisition; Data curation, Project administration; Resources; Supervision; Validation; Visualization; Writing - review & editing

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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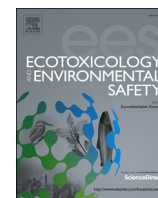
Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.ecoenv.2020.111321>.

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Comparison of Cd subcellular distribution and Cd detoxification between low/high Cd-accumulative rice cultivars and sea rice

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ABSTRACT

Salt-tolerant rice cultivar (sea rice) is a research hotspot worldwide due to its high yield in high salinity soil. However, knowledge regarding the cadmium (Cd) effects on the growth of sea rice is limited. To determine the short-term and long-term impact of Cd stress, relatively low/high Cd-accumulative rice cultivars and sea rice were grown to compare their growth responses to Cd stress over time. The results showed that sea rice presented the highest Cd concentrations in the root, stem, and leaves under 32-days of Cd stress. Cd stress shortened and thickened the rice root, and decreased the proportion of root diameters in the 0–0.2 mm range. Cd stress remarkably increased the Cd and Fe concentration in dithionite–citrate–bicarbonate (DCB) extracts, and the DCB–Cd and DCB–Fe concentrations were the highest in sea rice. The subcellular distribution of Cd in the rice roots indicated that Cd accumulated the most in the soluble fraction and cell wall. The contents of pectin and hemicellulose 2 in the root cell wall of the low-Cd accumulative rice variety CL755 were higher than those in MXZ and sea rice. Collectively, this work provides a general understanding of the Cd effects on sea rice growth and indicates that sea rice has a relatively high Cd accumulation compared with the other two rice cultivars. However, the specifically-related mechanism remains to be further studied.

1. Introduction

Heavy metals are widespread in natural and agricultural environments, and Cd is among the most toxic heavy metals that harms human health (Song et al., 2015). Cd accumulation in farmland is attracting increasing attention due to its great toxicity and high mobility from soil to plants and further to human bodies (Vig et al., 2003). It is well documented that excessive Cd directly or indirectly destroys physiological processes in plants, such as respiration, transpiration, photosynthesis, oxidative stress, root elongation, nitrogen metabolism, and mineral nutrition, leading to plant growth retardation, leaf chlorosis and low biomass in plants (Sanità Di Toppi and Gabbriellini, 1999; Thu et al., 2017; Yang et al., 2015). Rice is a major staple cereal crop worldwide and feeds more than 50% of the population (Li et al., 2016). However, Cd pollution in paddy soil severely threatens the rice quality, and Cd-contaminated rice has become the main Cd exposure source in humans, posing a health risk (Hu et al., 2009). Consequently, many effective techniques are developed to lower Cd accumulation in rice,

such as agronomic practices or breeding low Cd accumulative rice cultivars (Honma et al., 2016). Therefore, it is meaningful to distinguish the Cd accumulative characteristics and growth responses of different rice varieties.

The tolerance of plants to Cd stress determines the Cd accumulation potential of plants and the physiological response to Cd toxicity (He et al., 2008). In general, plants have evolved a series of defence mechanisms against Cd stress, such as Cd exclusion, restricted distribution of metal in sensitive tissues, binding metals to cell walls, chelation of organic molecules, and subcellular distribution of Cd (Hou et al., 2013; Wang et al., 2008). Outside the protoplast, metals can be accumulated by AM fungi, chelated in the rhizosphere, blocked in iron plaques, and bounded by a cell wall (Fu et al., 2016; Krzesłowska, 2011). However, after entering the roots, Cd is distributed among the subcellular fractions and combines with substances in plant cells, such as metallothioneins, phytochelatin, organic acids, etc. (Xin et al., 2017). Many investigations demonstrated that cell wall deposition, metal-chelating compounds (matrix polysaccharides) and vacuolar

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compartmentation play key roles in Cd detoxification and tolerance in plants (Xue et al., 2014; Yu et al., 2012).

Sea rice has recently become popular in China because it introduces new horizons to solve the food shortage problem (Chen et al., 2017). Considerable studies show that rice plants mainly tolerate salt by two mechanisms, i.e., ion repellency and osmotic tolerance (including tissue tolerance) (Roy et al., 2014). Tissue tolerance involves Na^+ separation in vacuoles, producing compatible solutes and enzymes that catalyse detoxification (Reddy et al., 2017). Interestingly, it has been suggested that rice heavy metal detoxification and salt tolerance mechanisms share some common characteristics, such as antioxidant systems, intracellular compartments and osmoprotectants in osmotic adjustment systems (proline, glycine betaine, etc.) (Ashraf et al., 2015; Fu et al., 2011; Yu et al., 2017a). However, to the best of our knowledge, the accumulative characteristics of heavy metals in salt-tolerant rice cultivars are rarely studied. In our previous study (unpublished), we surprisingly found that a salt-tolerant rice cultivar (HH12, with a salt tolerance of approximately 0.6% soil salinity) possessed a relative high Cd accumulative feature. Therefore, we further used three different rice cultivars to compare their root morphological change, Cd subcellular distribution and Cd detoxification under Cd stress.

2. Materials and methods

2.1. Plant cultivation

The rice (*Oryza sativa* L.) varieties used in these experiments included C Liangyou 755 (CL755), Mei Xiangzhan (MXZ) and Hai Hong 12 (HH12, a salt-tolerant rice variety) and were provided by the Institute of Subtropical Agriculture of Chinese Academy of Sciences and Guangdong Ocean University. CL755 possessed a relatively low Cd accumulative characteristic in grains (Cd concentration was approximately 0.23 mg/kg), while MXZ had a relatively high Cd accumulative characteristic in grains (Cd concentration was approximately 0.75 mg/kg), and both varieties were selected based on previous experiments. The rice seeds were surface-sterilized by soaking in 30% H_2O_2 for 15 min, followed by rinsing with deionized water and incubation in containers with moistened sands for germination. Seed germination was conducted in a temperature-controlled incubator (28/25 °C, 16/8 h, day/night) with a relative humidity of 70% for approximately 30 days. These seedlings were further placed in 250 ml beakers (containing 150 ml of 50% Yoshida's solution) for revival. The composition of Yoshida's solution was as follows: NH_4NO_3 1.43; CaCl_2 1.00; MgSO_4 1.64; K_2SO_4 1.00; NaH_2PO_4 0.32; FeCl_3 3.6×10^{-2} ; MnCl_2 9.4×10^{-3} ; H_3BO_3 1.9×10^{-2} ; $(\text{NH}_4)_6\text{Mo}_7\text{O}_{24}$ 5.17×10^{-4} ; ZnSO_4 1.52×10^{-4} ; CuSO_4 1.36×10^{-4} ; and Na_2SiO_3 5.00×10^{-3} (mM). All beaker walls were covered with black paper to avoid exposing the root to light. The pH of the nutrient solution was renewed every week, and the pH was adjusted to 5.0–5.2 every three days with 1 M sodium hydroxide (NaOH) or 1 M hydrochloric acid (HCl).

2.2. Experimental design

The hydroponic experiment was conducted in a growth chamber at the College of Agriculture, South China Agricultural University. The conditions were as follows: 28 °C during the day and 25 °C during the night. The humidity was approximately 75%, and the illumination intensity was approximately 3000 lx (light: dark photoperiod of 12 h: 12 h). After 7 days of growth in 50% Yoshida's solution, uniform and healthy seedlings of each rice variety were transplanted to 100% Yoshida's solution with or without 2 mg/L Cd^{2+} (supplied as $\text{CdCl}_2 \cdot 5/2\text{H}_2\text{O}$) for 32 days (32-d Cd stress). The short-time Cd stress began exactly 10 days before the rice harvest (10-d Cd stress), and the rice plants were transplanted from Cd-free containers to the other containers with a concentration of 2 mg/L Cd^{2+} . Each hydroponic container (diameter = 34 cm, height = 24.8 cm) contained 12 L nutrient

solution and 4 rice seedlings. Yoshida's solution was renewed every week, and the pH was adjusted to 5.0–5.2 every three days with 1 M NaOH or 1 M HCl.

2.3. Sampling and analysis

2.3.1. Determination of Cd content in the plants

The rice plants were harvested during the tillering stage (64 days after sowing). One rice plant was selected from each pot for the biomass and Cd content measurements. Different parts of the rice plant (leaf, stem, and root) were separated, and the roots were rinsed successively with tap water and distilled water to remove the nutrient solution. Fresh roots were scanned by a root scanner (Epson Expression 1600 pro, Model EU-35, Japan) and analysed by WinRHIZO Reg2009 to acquire the root parameters. The fresh biomass of the different rice organs was measured and then dried at 75 °C until a constant weight was attained. The dried samples were crushed and digested by microwave digestion before detection. Additionally, the quality control (CDHK-GBW(E) 100349, Certified reference material for the chemical composition of rice flour) and the blank samples were generated. The total Cd content was detected by graphite furnace atomic absorption spectrometry (Z700P, Jena, Germany).

2.3.2. Extraction and determination of Fe and Cd on the root surface

Another rice plant from each pot was harvested for the determination of Fe on the root surface. The iron plaques deposited on the root surface were extracted using the dithionite–citrate–carbonate (DCB) method (Chen et al., 2018). Briefly, parts of the fresh roots were incubated for 1 h with 50 ml of a solution containing 0.03 M sodium citrate ($\text{Na}_3\text{C}_6\text{H}_5\text{O}_7 \cdot 2\text{H}_2\text{O}$), 0.125 M sodium bicarbonate (NaHCO_3), and 1.0 g sodium dithionite ($\text{Na}_2\text{S}_2\text{O}_4$). After filtration, the extraction solution was diluted in 100 ml volumetric flask with ultrapure water, and then, the sample was analysed to detect the Fe and Cd concentrations in the iron plaques using AAS (Z700P, Jena, Germany). The fresh roots used for the Fe extraction were then oven-dried and weighed to calculate the DCB-Cd and DCB-Fe concentrations.

2.3.3. Extraction and determination of Cd on the root subcellular cell

The fresh roots (after Fe extraction) were soaked in 25 mM $\text{Na}_2\text{-EDTA}$ solution for 15 min to remove the Cd adsorbed on the root surface and rinsed thoroughly with deionized water. Then, 0.5 g fresh roots were homogenized using a pre-cooling mortar and pestle in a medium containing 20 ml ice-bathed extraction buffer [0.25 M sucrose, 50 mM Tris-HCl (pH 7.5), and 1 mM dithioerythritol] (Weigel and Jäger, 1980). All steps were performed at 4 °C. The resulting brei was centrifuged at 5000 g for 15 min. In addition, the resulting pellet contained mainly cell walls and cell wall debris and was designated the cell wall fraction (I). The supernatant obtained from the first centrifugation step was then centrifuged at 20,000 g for 45 min to sediment the cell organelles. The pellet was designated an organelle fraction (II). The resultant supernatant solution was designated the soluble fraction (III). All fractions were digested by microwave digestion before detection.

2.3.4. Cell wall polysaccharide extraction and Cd determination

After soaking in 25 mM $\text{Na}_2\text{-EDTA}$ solution, 0.5 g fresh roots were weighed and rinsed twice with ice-cold distilled water. Then, the weighed fresh roots were immediately ground using mortar and pestle in liquid N_2 . Then, the powder was used for the fraction collection of the cell wall, pectin, hemicellulose 1 (HC1), and hemicellulose 2 (HC2) based on a previously described method (Zhong and Lauchli, 1993). All fractions were digested by microwave digestion before detection. The total Cd content was detected by graphite furnace atomic absorption spectrometry (Z700P, Jena, Germany).

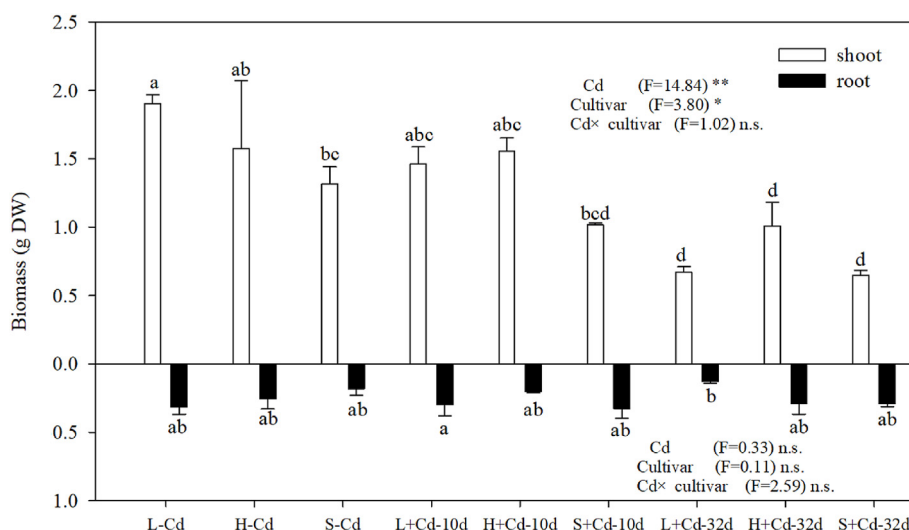


Fig. 1. Biomass of different rice varieties.

2.4. Statistical analysis

The statistical difference analysis was performed using a one-way ANOVA and two-way variance (SAS 9.0, USA). The figures were generated using SigmaPlot 10.0 (Systat Software, Inc, USA). All data are presented as the mean \pm standard deviation ($n = 3$).

3. Results

3.1. Biomass of different rice varieties

Cd stress significantly affected rice biomass accumulation, especially the above-ground biomass (Fig. 1). With 10-d Cd stress, the shoot biomass of CL755, MXZ and HH12 was slightly decreased by 23.1%, 1.3%, and 22.9%, respectively. However, under the 32-d Cd stress, the aboveground biomass of the different rice cultivars was significantly decreased. The aboveground biomass of CL755, MXZ and HH12 was decreased by 64.9%, 36.1%, and 50.8%, respectively. Regarding the root biomass, Cd had no notable effect on the root biomass of MXZ and HH12. However, compared with the 10-d Cd stress, the root biomass of CL755 was significantly decreased by 59.3% under the 32-d Cd stress. Based on the ANOVA, the Cd levels, rice cultivars and Cd \times cultivar interaction presented no significant effects on the root biomass, while the Cd levels and cultivars significantly affected the biomass accumulation in the shoot.

L: C Liangyou 755; H: Mei Xiangzhan; S: Hai Hong 12. “-Cd” denotes no Cd stress, “+ Cd-10d” denotes 10-day Cd stress; “+ Cd-32d” denotes 32-day Cd stress. *($p < 0.05$); **($p < 0.01$); n.s. denotes no significant difference. All values are presented as the mean \pm standard error ($n = 3$), and bars with different letters indicate significant differences ($p < 0.05$).

3.2. Root morphological change

The Cd stress obviously shortened and thickened the rice root and reduced the number of lateral roots (Fig. 2a). Additionally, the Cd stress significantly decreased the total root length in the different rice varieties (Fig. 2b). It was found that the total root length of CL755 was markedly decreased under the 10-d and 32-d Cd stress by 34.8% and 48.1%, respectively. While the total root length of MXZ and HH12 presented no significant decrease under the 10-d Cd stress. However, a reduced root length was observed in MXZ and HH12 under the 32-d Cd stress (reduction by 33.4% and 32.6%, respectively). In addition, based on the ANOVA, the Cd levels notably affected the total root length in all

rice varieties ($p < 0.05$).

The number of root diameters in the 0–0.2 mm range was the largest, accounting for approximately 59%–71%, followed by the number of root diameters in the 0.2–0.5 mm range (approximately 11%–21%), 0.5–1.0 mm range (approximately 13%–17%) and ≥ 1.0 mm (approximately 1%–2%). Moreover, in the presence of Cd, the root classification based on diameter showed that the root tended to become thicker under the 32-d Cd stress (Fig. 2c). Noticeably, the proportion of root diameters in the 0.2–0.5 mm range increased by 7.2–21.1%. However, root lengths in a diameter range of 0–0.2 mm sharply decreased by 35.6–51.0%. The proportion of root lengths in a diameter range of 0–0.2 mm remarkably decreased in CL755 and MXZ, while the percentage of root lengths in the diameter range of 0.2–0.5 mm significantly increased in MXZ.

3.3. DCB-Cd and DCB-Fe concentrations in iron plaques

The Cd and Fe concentrations in the DCB extracts from different rice cultivars were significantly affected by the Cd stress (Fig. 3a and b). The content of both DCB-Cd and DCB-Fe bounded on the root surface gradually increased over time. There was no remarkable difference in the DCB-Cd and DCB-Fe concentrations among the different rice varieties under the 10-d Cd stress. However, the quantitative estimation of the metals showed that HH12 presented the highest DCB-Cd and DCB-Fe concentrations on the root surface, representing an increase of 78.8% and 52.0%, respectively, compared with that observed following the 10-d Cd stress. In addition, CL755 exhibited the lowest DCB-Cd and DCB-Fe concentrations on the root surface. The correlation analysis demonstrated that DCB-Cd and DCB-Fe were not positively related when treated with the 10-d Cd stress ($r = 0.3344$, $F = 0.88$, $p > 0.05$). However, a significant positive correlation was observed between DCB-Cd and DCB-Fe with the 32-d Cd stress ($r = 0.8541$, $F = 18.88$, $p < 0.05$).

3.4. Cd content in plant tissues of different rice varieties

The Cd concentrations in the different rice varieties showed the trend of root > stem > leaves under the -Cd and +Cd treatments (Fig. 4). Without Cd stress, no notable difference in the Cd concentration was observed in the different parts among the rice varieties. However, under the 10-d Cd stress, the Cd concentration in the root of MXZ was the highest, followed by CL755 and HH12 (Fig. 4a). However, the Cd concentration in the shoot presented a different tendency. The highest Cd concentrations in the stem and leaves were observed in

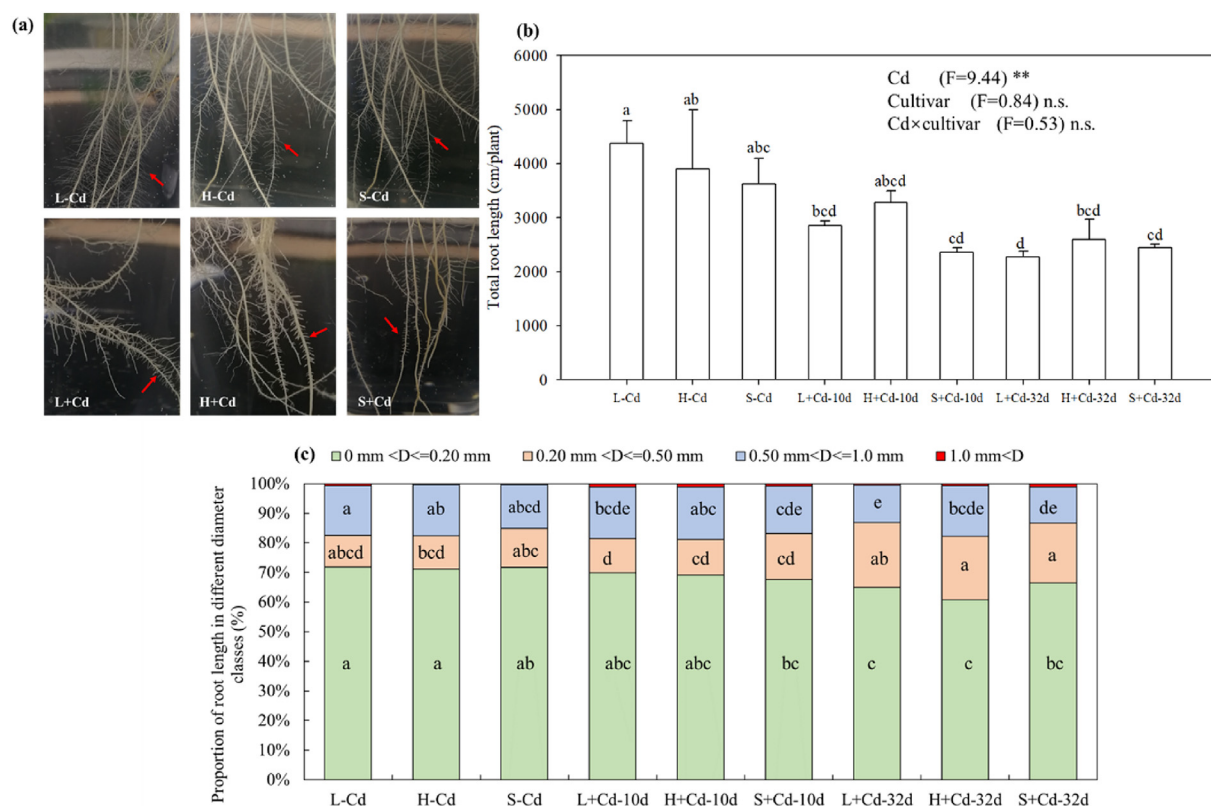


Fig. 2. Total root length and root diameter classes in different rice varieties. (a) phenotypic characterizations of partial roots of different rice varieties; (b) total root length; (c) proportion of root diameter classes. L: C Liangyou 755; H: Mei Xiangzhan; S: Hai Hong 12. “-Cd” denotes no Cd stress, “+Cd-10d” denotes 10-day Cd stress; “+Cd-32d” denotes 32-day Cd stress. * ($p < 0.05$); ** ($p < 0.01$); n.s. denotes no significant difference. All values are presented as the mean \pm standard error ($n = 3$), and bars with different letters indicate significant differences ($p < 0.05$).

HH12 at 10.12 and 5.0 mg/kg, respectively. Moreover, under the 32-d Cd stress, the Cd concentrations in the root, stem, and leaves of HH12 were the highest, followed by MXZ and CL755. Noticeably, the Cd concentration in the stem of HH12 was up to 160.5 mg/kg, which was 4.4-fold and 3.3-fold higher than the Cd concentration in the stem of MXZ and CL755, respectively (Fig. 4b).

Similarly, the Cd accumulation in the different rice cultivars abided by the order of HH12 > MXZ > CL755 in both the root and shoot (Fig. 4d). The Cd accumulation in the different rice varieties presented no significant difference without the Cd stress. Under the 10-d Cd stress, the Cd accumulation in the shoot of HH12 was the highest, while the Cd content in the root presented no marked difference among the different rice varieties. Additionally, the Cd accumulation in HH12 was the highest in both the root and shoot under the 32-d Cd stress, followed by

MXZ and CL755. In addition, the Cd accumulation in the shoot of HH12 was approximately 168.99 $\mu\text{g}/\text{plant}$, which was 3.08-fold and 2.88-fold higher than the Cd accumulation in the shoot of CL755 and MXZ, respectively.

3.5. Subcellular distribution of Cd in the root

Cd accumulated in subcellular fractions was observed to exhibit a trend of soluble fraction > cell wall > organelle (Fig. 5). The Cd concentration in the soluble fraction was considerably greater than that in the cell wall and cell organelle fractions. Overall, the percentage of the cell wall, organelle, and soluble fractions were 19.4–30.6%, 5.1–10.7% and 64.1–71.4%, respectively. Despite the high Cd concentration, there was no significant difference in the Cd concentration

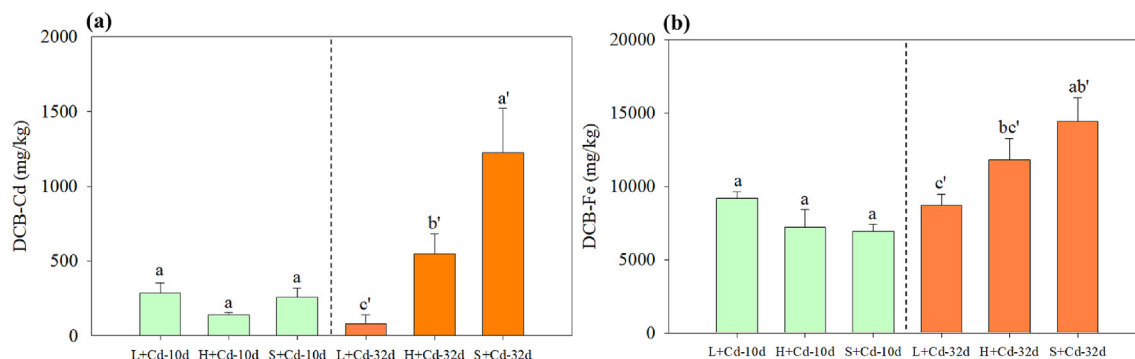


Fig. 3. Cd and Fe contents in the iron plaque in different rice varieties. (a) DCB-Cd content; (b) DCB-Fe content. L: C Liangyou 755; H: Mei Xiangzhan; S: Hai Hong 12. “+Cd-10d” denotes 10-day Cd stress; “+Cd-32d” denotes 32-day Cd stress. The DCB-Cd and DCB-Fe concentrations were based on the root dry weight. All values are presented as the mean \pm standard error ($n = 3$), and bars with different letters indicate significant difference ($p < 0.05$).

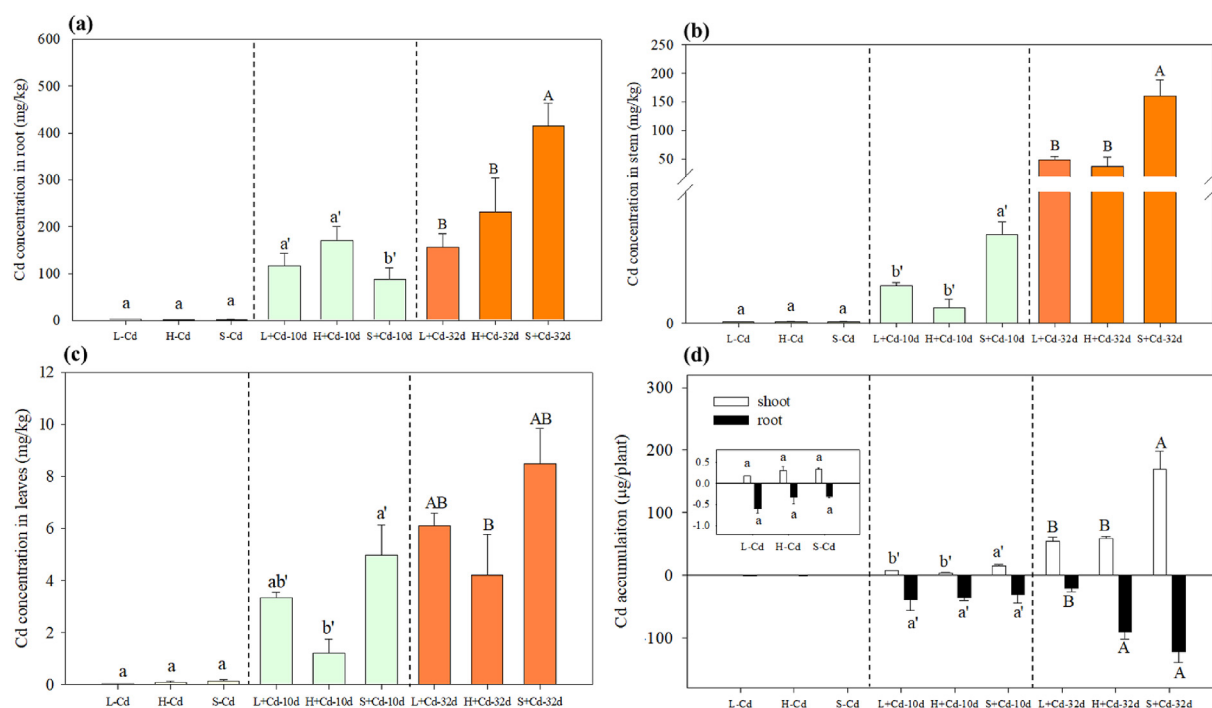


Fig. 4. Cd content in plant tissues of different rice varieties. (a) Cd concentration in the root; (b) Cd concentration in the stem; (c) Cd concentration in the leaves; (d) Cd accumulation in different rice varieties. L: C Liangyou 755; H: Mei Xiangzhan; S: Hai Hong 12. “-Cd” denotes no Cd stress, “+Cd-10d” denotes 10-day Cd stress; “+Cd-32d” denotes 32-day Cd stress. All values are presented as the mean \pm standard error ($n = 3$), and bars with different letters indicate significant differences ($p < 0.05$).

in the organelle fraction over time. However, the Cd concentration in the cell wall and soluble fractions under the 32-d Cd stress was greatly increased compared with that under no Cd stress or 10-d Cd stress. Compared with the 10-d Cd stress, the Cd concentration in CL755, MXZ and HH12 under the 32-d Cd stress was increased by 44.8%, 35.6%, and 58.7%, respectively, in cell wall fraction and 52.2%, 18.3%, and 51.6%, respectively, in the soluble fraction. Furthermore, the total Cd concentration in the subcellular region was the highest in HH12, followed by CL755 and MXZ. Based on the ANOVA, the Cd levels notably affected the Cd subcellular distribution among the different rice varieties ($p < 0.01$).

L: C Liangyou 755; H: Mei Xiangzhan; S: Hai Hong 12. “-Cd” denotes no Cd stress, “+Cd-10d” denotes 10-day Cd stress; “+Cd-32d” denotes 32-day Cd stress. *($p < 0.05$); **($p < 0.01$); n.s. denotes no

significant difference. The values of Cd concentration (mg/kg) for different fractions are derived by using fresh weight bases. All values are presented as the mean \pm standard error ($n = 3$), and bars with different letters indicate significant differences ($p < 0.05$).

3.6. Cell wall polysaccharide content and Cd concentration

The total polysaccharide content in CL755 was notably higher than that in MXZ and HH12 under the 32-d Cd stress (Fig. 6a). One of the polysaccharide components, i.e., hemicellulose 1, presented no differences among the rice varieties under the Cd stress over time. In contrast to hemicellulose 1, the content of hemicellulose 2 in CL755 under the 32-d Cd stress was the highest at 1.97 and 1.96 times that in MXZ and HH12, respectively. In addition, the pectin content was also the highest

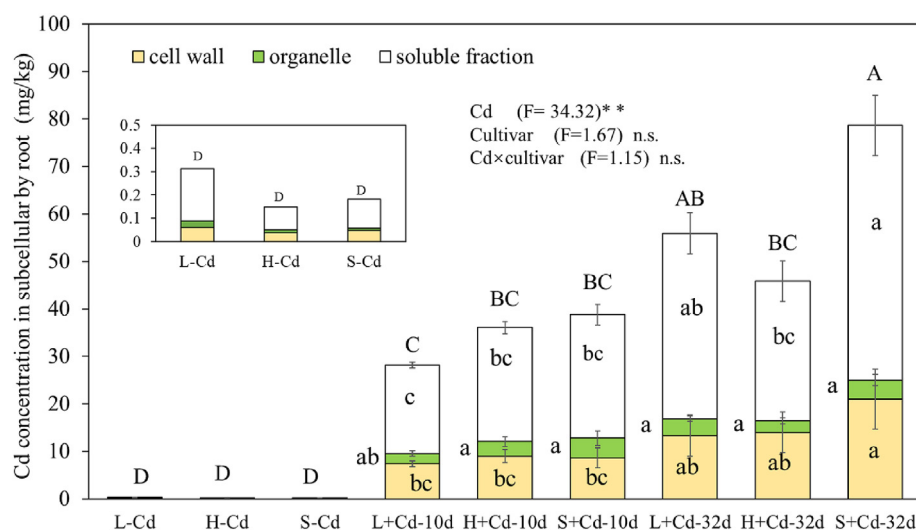


Fig. 5. Subcellular distribution of Cd in the root of different rice varieties.

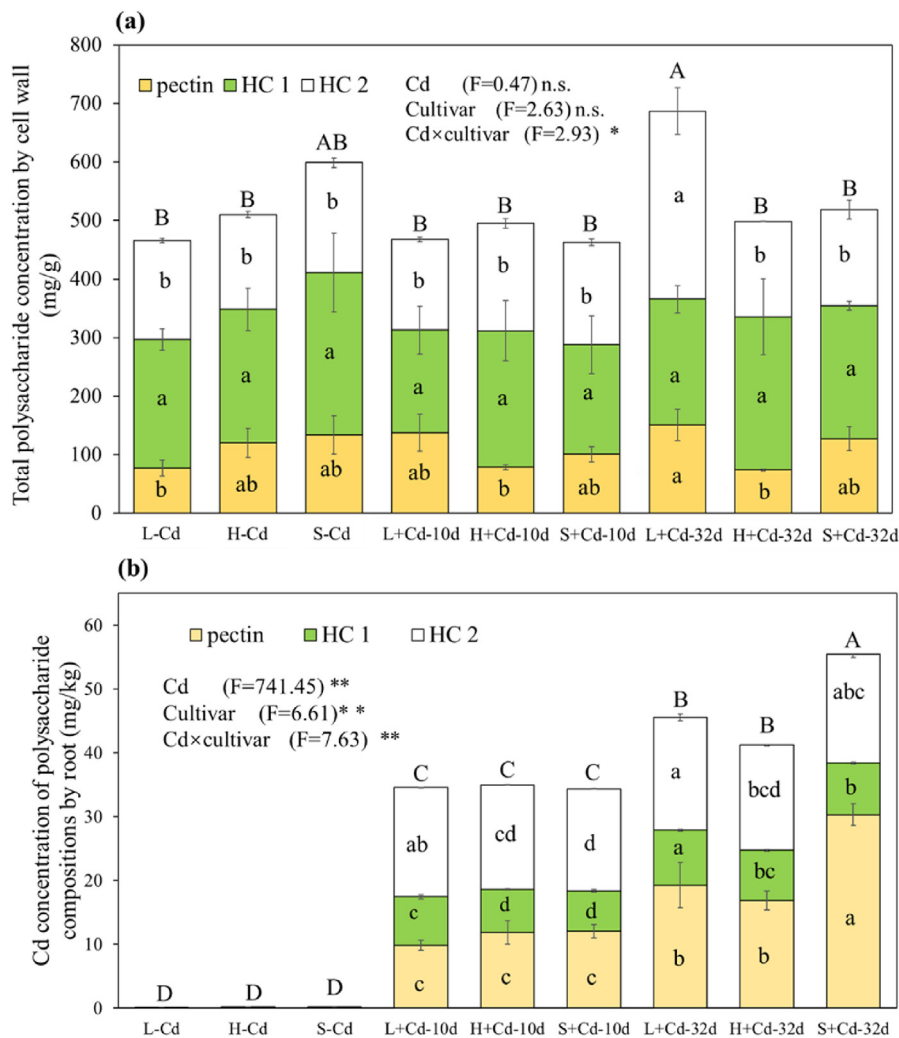


Fig. 6. Cell wall polysaccharide content and Cd concentration in different rice varieties.

in CL755, followed by HH12 and MXZ. Based on the ANOVA, the Cd × cultivar interaction presented significant effects on the polysaccharide concentration on the root cell wall ($p < 0.05$).

The Cd concentration in cell wall polysaccharide fractions was remarkably affected by the Cd levels and rice cultivars (Fig. 6b). The Cd concentrations in pectin, hemicellulose 1 and hemicellulose 2 were significantly increased under the 32-d Cd stress, and HH12 contained the highest total Cd concentration in cell wall polysaccharides. Under the 10-d Cd stress, the Cd concentration in pectin among the different rice varieties showed no difference; however, the hemicellulose 1 and 2 concentrations were significantly higher in CL755 than those in the other varieties under the 32-d Cd stress. Furthermore, the Cd concentration in pectin in HH12 was the highest under the 32-d Cd stress (1.6 and 1.8-fold higher than that in CL755 and MXZ). Additionally, the Cd concentration in hemicellulose 1 and hemicellulose 2 in CL755 was remarkably the highest, followed by HH12 and MXZ under Cd stress. Based on the ANOVA, the Cd levels, rice cultivars and Cd × cultivar interaction all showed marked effects on the Cd concentration on polysaccharide fractions.

L: C Liangyou 755; H: Mei Xiangzhan; S: Hai Hong 12. “-Cd” denotes no Cd stress, “+ Cd-10d” denotes 10-day Cd stress; “+ Cd-32d” denotes 32-day Cd stress. HC 1: hemicellulose 1; HC 2: hemicellulose 2. * ($p < 0.05$); ** ($p < 0.01$); n.s. denotes no significant difference. The values of Cd concentration (mg/kg) for different fractions are derived by using fresh weight bases. All values are presented as the mean ± standard error ($n = 3$), and bars with different letters indicate

significant differences ($p < 0.05$).

4. Discussion

Cd is among the most toxic heavy metals, and excessive Cd seriously destroys plant growth by disturbing plant physiology, nutrient homeostasis, enzymatic reactions, photosynthetic pigments, etc. (Rehman et al., 2017; Yang et al., 2015). Cd stress could gradually threaten the growth of plants and stress chlorosis in plant leaves (Liu et al., 2008; Ultra et al., 2016). In this experiment, the addition of Cd greatly affected the shoot biomass accumulation in rice plants. These results were consistent with previous studies (Zhang et al., 2008).

Generally, root elongation inhibition is regarded as the first evidence of Cd toxicity in plants (Munzuroglu and Geckil, 2002). It has been well established that cell division at the root tips and cell elongation in the extension zone were affected by toxic heavy metals (Bonifacio and Montano, 1998; He et al., 2010). In this study, a reduced root length were found with high Cd stress, which is consistent with previous studies. In addition, the results indicated that the root of CL755 was the most sensitive to Cd stress and was most obviously shortened. Meanwhile, the 0–0.2 mm diameter range accounted for the largest majorities of root diameter proportions but decreased significantly with a relative increase of 0.2–0.5 mm under the 32-d Cd stress. However, Li et al. (2009) demonstrated that the specific root length of the hyperaccumulating ecotype was mainly constituted by roots with diameters between 0.2 and 0.4 mm under a high toxic level

of heavy metals. In addition, Lu et al. (2013) illustrated that Cd-induced decreases in root length and root surface area are significant in the 0–0.2 and 0.2–0.4 mm diameter classes of peanut cultivars. It has been suggested that coarse roots could retain more Cd in the tissue and, consequently, reduce Cd transfer from the root to shoots (Yu et al., 2017b), while longer root or more fine roots could have higher Cd uptake (Huang et al., 2015, 2019; Lu et al., 2013). In this study, three rice cultivars showed no significant difference in the root length, root surface area and root diameters under Cd stress, indicating that a remarkable Cd transportation rate may be the dominating factor responsible for the marked difference in Cd accumulation in rice plants.

Iron plaques are considered to have a great affinity to heavy metals (e.g., Pb, As and Cd) and act as a barrier to heavy metal ions (Cao et al., 2018a; Li et al., 2019; Sebastian et al., 2016; Yang et al., 2016). Although the root morphology change presented no marked difference among the rice cultivars, the iron plaque was found to notably increase under the high Cd stress. In addition, a significant positive correlation was observed between the DCB-Cd and DCB-Fe contents under the 32-d Cd stress, indicating that the iron plaque content increased to restrain more Cd content. However, although the iron plaque in the three rice varieties was significantly increased (especially in HH12) in the case of high Cd stress, it did not prevent extremely high Cd accumulation in the rice plants (Fig. 4d). These results imply that the blocking effects of the iron plaque were limited under the high Cd toxicity condition and that the iron plaque on root surfaces has minimal impact in affecting the uptake and accumulation of Cd by rice plants (Liu et al., 2008).

After entering the plant roots, Cd tends to be distributed in the inactive area of the root (the vacuole or apoplast) to mitigate damage caused by Cd stress (Boominathan and Doran, 2003; Zhang et al., 2014). In this study, Cd in the rice roots was distributed by the order of soluble fraction > cell wall fraction > organelle fractions (Fig. 5), which is consistent with the Cd subcellular distribution in tea plant (*Camellia sinensis*) and pokeweed (*Phytolacca americana* L.) (Cao et al., 2018b; Fu et al., 2011). However, some other studies demonstrate that the root cell walls are the main binding sites of Cd, followed by the soluble fraction and organelle fractions, such as in watercress (*Nasturtium officinale* L. R. Br.) and soybean cultivars (Wang et al., 2015, 2016). However, our results indicated that the proportion of Cd in the soluble fraction and cell wall fraction significantly increased with the prolongation of Cd stress. In addition, a large percent of Cd (67.4%) was found to distribute in the soluble fraction, which was mainly composed of vacuoles (Zhuo et al., 2017). It has been reported that vacuolar compartmentalization plays an important role in reducing free Cd in the cytosol. In addition, phytochelatins (PCs) and glutathione (GSH) are the main metal-ligand molecules involved in vacuolar Cd sequestration (Shahid et al., 2017). Cd²⁺ can be chelated by the SH groups of PCs due to the high affinity of the thiol groups to Cd (Akhter et al., 2012).

Polysaccharides (including cellulose, hemicellulose, pectin, and protein) are the main components of plant cell walls (Krzesłowska, 2011). Polysaccharides contain different chemical functional groups (-COOH, -SH, etc.), which can effectively bind metal cations and affect the accumulation of Cd in the cell wall (Zhao et al., 2019). In this study, we demonstrated that the pectin and hemicellulose 2 contents were considerably the highest in CL755 (Fig. 6a). Pectin and hemicellulose are important for Cd tolerance in plant cell walls (Xiong et al., 2009), suggesting that CL755 may have more polysaccharide functional bases to bind cadmium ions. In addition, the results obtained by Zhao et al. (2019) indicate that Se addition increased the contents of pectin and hemicelluloses 2 in the root cell wall of oilseed rape. When treated with Cd stress, the Cd content binding to hemicellulose 1 and hemicellulose 2 were both the highest in CL755. However, the total Cd concentration in the polysaccharide composition was the highest in HH12 (Fig. 6b), suggesting that the Cd compartmentalizing capacity of the cell wall is insufficient when affected by high and long-term Cd stress (Wang et al., 2015).

In this study, Cd was mainly concentrated in the rice root, followed

by the stems and leaves (Fig. 4). Although the rice plants were exposed to a high Cd concentration, the Cd concentration in the leaves remained low (Yang et al., 2018). Noticeably, the highest Cd concentration and Cd accumulation were observed in HH12, and the Cd concentration in the stem of HH12 was even as high as 160.5 mg/kg. As one of the main characteristics of hyperaccumulating plants (Cd only), the minimum concentration of Cd in the shoot of a hyperaccumulator plant is 100 mg/kg (Wei et al., 2006). It has been reported that both salinity and heavy metal stress can elicit the function of the antioxidant system, the osmoprotectant proline, the intracellular compartments, etc. In addition, Ma et al. (2013) found that NaCl pretreatment increased rice tolerance to Cd stress. Mekawy et al. (2018) revealed that the *OsMT-3a* gene from rice confers tolerance not only to salinity but also to heavy metal stress by detoxifying the ROS generated by these stresses. However, the mechanisms related to the relatively high Cd-accumulative traits of sea rice (HH12) need further investigation.

5. Conclusion

In summary, the Cd concentration and Cd accumulation in different rice cultivars abided by the order of HH12 > MXZ > CL755, suggesting that sea rice possessed a relatively high Cd accumulative features. A high Cd concentration was observed to significantly affect biomass accumulation and shortened and thickened the rice root in all rice varieties. Although Cd stress notably enhanced iron plaque formation and increased the Cd subcellular distribution in the soluble fractions and cell walls, it did not ameliorate Cd accumulation in the plants. Higher pectin and hemicellulose contents were found in the root cell walls of CL755, indicating that cell wall polysaccharide likely plays an important role in alleviating Cd toxicity. Collectively, this work generally studied high Cd stress effects on the growth of sea rice, and these results indicate that sea rice showed a relatively higher Cd accumulation under 32-d Cd stress, but the related mechanism in sea rice needs to be further studied.

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Effect of rainwater-borne hydrogen peroxide on manure-derived Cu and Zn speciation distribution and bioavailability in rice-soil system

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ABSTRACT

Pot experiment was conducted to examine the effects of rainwater-borne hydrogen peroxide (H_2O_2) on transformation of Cu, Zn from pig manure in soils and its resulting impacts on the growth of Cu and Zn uptake by a rice plant. Results showed that the exogenous application of H_2O_2 significantly improved the rice biomass and yield. Addition of H_2O_2 into the soils led to reduced uptake of soil-borne Cu and Zn by the rice plants and this had a significant effect on reducing the accumulation of Zn in rice grains. It was indicated that the increased pH in soil might play important role in reducing Cu and Zn content in rice. Furthermore, Cu and Zn content in exchangeable form and carbonate bounded form dramatically decreased in soil, on the contrary, the organic combination state increased significantly in H_2O_2 treatment. The findings point to a potential research direction that rainwater-borne H_2O_2 in nature may help to change morphology of heavy metals in natural soil environments, but further study is still needed to explore the related mechanisms in Cu and Zn in manures and paddy rice field receiving rainwater-borne H_2O_2 .

1. Introduction

Global agriculture is facing unprecedented challenges and risks, with substantial environmental costs, including emissions of greenhouse gases, biodiversity loss, environmental pollution and degradation of land and freshwater (Chen et al., 2014). The zero growth of chemical fertilizers in China and the utilization of livestock and manure resources have become a major trend to pursue more grain with lower environmental costs. However, China has become one of the biggest pork-producing countries in the world (Xu et al., 2013), and the number of pigs even exceeded 0.68 billion in 2016 (Qian et al., 2018). As a consequence, considerable amount of pig manures emerge and has been encouraged to be excellent organic fertilizers to field crops in China (Liang et al., 2018; Wang et al., 2018). Furthermore, the abuse of microelement additive, such as Cu and Zn, causes generally high concentrations of Cu and Zn in manures (Zhang et al., 2012a,b; Wang et al., 2013). Many investigations indicated that Cu and Zn levels in pig manures were remarkably higher than other animal manures (Sager, 2007; Shi et al., 2011; Zhang et al., 2012b; Xu et al., 2013; Zhao et al., 2014; Liang et al., 2017a,b). And consequently, the successive and long-term applications of livestock waste result in serious heavy metal or

antibiotic pollution in soil (Peng et al., 2015; Meng et al., 2018). It is reported that the Cu and Zn levels in pig manures generated from intensive farming were found to be 1726 and 1506 mg/kg, respectively in China (Cang et al., 2004). And the mass balance modeling indicates that Cu and Zn will exceed the threshold levels for agricultural soils in China in the next 10–50 years, with annual and successive application of pig manures (Qian et al., 2018).

Rice (*Oryza sativa* L.) is one of the most important cereal crops in the world, feeding more than 50% population worldwide (Li et al., 2016), and China is the second-largest rice cultivation area and the highest rice production in the world. It is estimated that rice-growing area in China are one of the biggest potential sites for manure application (Liang et al., 2017a,b). High animal density is generally accompanied by production of a surplus of manure, causing threats for soil heavy metals or antibiotic pollution (Peng et al., 2015). Therefore, it is crucial to evaluate effects of the manure application on the rice growth and its heavy metal accumulations, and meaningful to reduce the heavy metal contents in brown rice to obtain a sustainable food-secure future in China.

Atmospheric deposition is considered to be a major process that removes pollutants from the atmosphere and an important source of

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nutrients and contaminants for ecosystems. And the ability of the atmosphere to remove pollutants through oxidation is known as atmospheric oxidation, which is one of the most important natural features of the atmosphere (Pan and Wang, 2015). Many oxidants involved in the tropospheric atmospheric chemical processes, such as ozone, OH radicals, peroxy radicals (HO_2 and RO_2), peroxides (H_2O_2 and ROOH), etc. (Prinn, 2003; Lin et al., 2019). H_2O_2 , one of the important atmospheric oxidants, is commonly found in natural water or rainwater, with a molar concentration frequently ranging from 5 to $100\ \mu\text{M}$ (Goncalves et al., 2010; Qin et al., 2017a,b). Except the oxidation ability to remove pollutants in atmosphere, it is also reported that H_2O_2 in natural water closely relate to photochemical reaction and redox reaction, which being a key factor to affect the migration, transformation, fate and ecological effects of many substances (such as metal ions, herbicides, humus, etc.) in water environment (Cooper and Lean, 1989; Qin et al., 2016). For example, previous pieces of work in our study showed that H_2O_2 at a concentration range frequently encountered in rainwater could lead to degradation of diuron, butachlor and glyphosate (Qin et al., 2013, 2017). Furthermore, it has been demonstrated that Fenton reaction mediated by H_2O_2 had significant effects on affecting As transformation (Ma et al., 2014) and reducing uptake of soil-borne As by rice plants (Qin et al., 2016). And it is indicated that H_2O_2 modified biochar could act as effective surface sorbent to remove heavy metals, such as Pb^{2+} , Cu^{2+} , Cd^{2+} and Zn^{2+} (Wang and Liu, 2018). But how would the atmospheric oxidant, H_2O_2 , affect the chemical speciation change, bioavailability of heavy metal (mainly Cu and Zn) of pig manures in soil are rarely studied.

Therefore, in this study, we used exogenous H_2O_2 application to imitate rainwater-borne H_2O_2 , and to explore the dose of H_2O_2 effects on Cu and Zn bioavailability derived from pig manure in soil and different parts of rice, trying to reduce Cu and Zn content in rice grain. This work help us obtain further insights into the potential effects of rainwater-borne H_2O_2 on alleviating Cu and Zn stress in rice, so as to provide theoretical basis to realize the harmless and resource utilization of livestock manure and to reduce the pollution risk of heavy metal in soil.

2. Material and methods

2.1. Materials

Rice (*Oryza sativa* L.) variety in this experiment was Huang Huazhan, provided by Rice Research Institute in Guangdong Academy of Agricultural Sciences. Acquired from the experimental field of the Ecology Department, the tested soil was dried naturally and sifted by 2 mm sieve, without any soil gravel, leaves, and other debris. The initial pH of soil was 6.19, the organic matter content was 16.06 g/kg, and the total N, P, K were 1.08, 0.96, 28.08 g/kg, respectively. The total Cu and Zn contents were 49.16 and 86.43 mg/kg. The dry-fermentation-bed pig manure was obtained from a pig farm near Guangzhou. After being dried, pig manures were all sifted by 2 mm sieve and uniformly mixed. The pH of pig manure was 6.29, and the organic matter content was 232.27 g/kg. The total N, P, K were 12.3, 9.15, 2.37 g/kg, and the total Cu and Zn contents were 0.34 and 0.91 g/kg, respectively. H_2O_2 (30%, guarantee reagent) used in this study was provided by Xilong Chemical co., LTD.

2.2. Experimental design

H_2O_2 effects on the morphological change, bioavailability of manure-derived Cu and Zn in soil-rice system was studied by a pot experiment. The tested soil was thoroughly blended with the pig manures (200 g per pot), with 65% field capacity for 1 week to stay homogeneous. The rice seeds were surface-sterilized by soaking in 30% H_2O_2 for 15 min, then were rinsed with deionized water and placed in containers with moistened sands for germination. After the

germination, six uniform healthy rice seedlings with 4 leaves were transplanted to each pot (diameter = 25 cm, height = 15 cm) with 5 kg paddy soil. Two weeks later, 250 mL H_2O_2 solution with different concentrations (20, 50, 100 μM) were sprayed every 2 days, according to the average annual rainfall in Guangzhou area (1800 mm, the evaporation rate is 40%). And same amount of deionized water was applied every 2 days, which served as CK, namely 0 μM H_2O_2 . Water surface was 1–2 cm higher than the soil surface during the growth process, and a compound fertilizer (15-15-15) was used to maintain N, P, K balance for rice growth. Each treatment had 4 replicates, and the harvest time was on the 125th day.

2.3. Sample preparation and analytical methods

Prior to starting the pot experiment, the pH, the organic matter content and total contents of N, P, K, Cu and Zn in soil and pig manures were measured. The soil pH was measured by a pH glass electrode in H_2O at a water: solid ratio of 2.5:1. The soil carbon content was determined by potassium dichromate oxidation method. The total N was determined by semimicro-kjeldahl method after the soil was digested by HF-HClO_4 , the total P was determined by acidic molybdate-ascorbic acid blue color method and the total K was determined by the flame photometry method (Bao, 2008).

After the rice harvest, the straw and root portions of rice plant were separated. The roots were rinsed with tap water and then with distilled water to remove the impurities attached to the sample surface. Fresh biomass of straw and root were collected, and oven-dried at 70 °C, until constant weight was attained. Soil samples were mixed and blended, and then collected from each pot. The samples of grain, straw and root were ground in a stainless steel mill and digested with concentrated $\text{HNO}_3\text{--HClO}_4$, the collected soil was air-dried and passed through a 2 mm sieve, and digested with concentrated $\text{HF-HNO}_3\text{--HClO}_4$. Different chemical speciation of Cu and Zn were extracted by the method of Tessier et al. (1979). Then the concentrations of Cu and Zn of soil and plant were measured by atomic absorption spectrometry (Z700P, Jena, Germany). At the same time, quality control and blank samples were made.

2.4. Statistical analysis

Statistical analysis of the experimental data was performed using one-way analysis of variance (ANOVA) and the means compared using significant difference (Duncan) method at 5% level (IBM SPSS software Version 17.0). All experimental data were presented as mean \pm standard error ($n = 4$).

3. Results and discussion

3.1. Soil pH and organic matter content

The application of H_2O_2 significantly increased the soil pH and the organic matter content when compared with CK. The initial pH was 5.96, then went up to 6.78, 6.57 and 6.31 respectively at H_2O_2 concentration of 20, 50 and 100 μM . Compared with CK, the soil organic matter content increased by 23.27%, 10.66% and 13.40%, respectively with H_2O_2 concentration of 20, 50 and 100 μM . The pH and organic matter content both increased most significantly under the treatment of 20 μM H_2O_2 (Fig. 1). Fenton or Fenton-like reactions can be triggered by the contact of Fe^{2+} in acid soil of southern China with H_2O_2 . The products include hydroxyl radical with high reactivity and $\text{Fe}(\text{OH})_3$ colloid, which improved soil pH value for its alkalinity (Hall and Silver, 2013). According to the Pearson analysis, the soil pH showed negative relation with Cu content in rice root, stem and leaves. As for Zn content in rice tissue, the soil pH was significantly negatively correlated with Zn content in rice root and the brown rice. These results indicated that soil pH might play important role in changing the Cu and Zn contents in rice

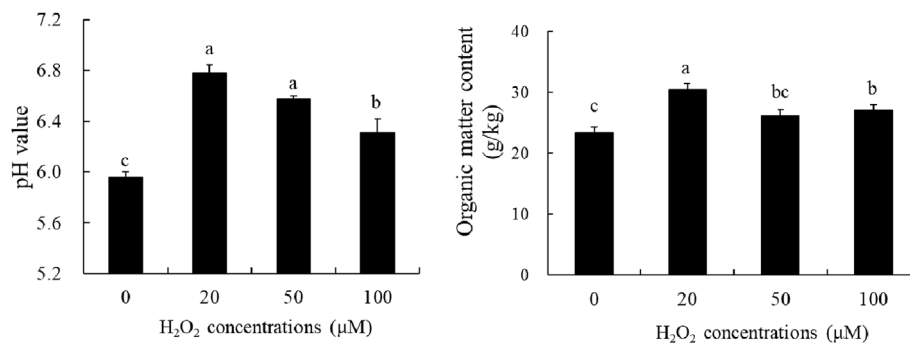


Fig. 1. Effects of H₂O₂ concentrations on soil pH and organic matter content. All values are presented as mean \pm standard error (n = 4) and bars with different letters indicate significant difference among H₂O₂ concentrations ($p < 0.05$).

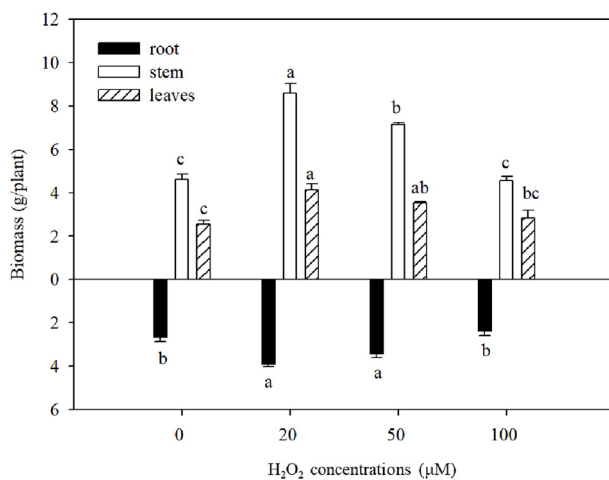


Fig. 2. Effects of H₂O₂ concentrations on rice biomass. All values are presented as mean \pm standard error (n = 4) and bars with different letters indicate significant difference among H₂O₂ concentrations ($p < 0.05$).

plant. Moreover, it has been addressed that the application of Fenton method can well remove the organic matter and color from different industrial wastewater (Bissey et al., 2006; Riaño et al., 2014). In the simplest case, H₂O₂ directly oxidizes organic compounds in a peroxidic-type reaction by a two-electron process without O₂ formation, which lead to organic carbon decomposition (Mikutta et al., 2005). And during the decomposition of organic matter, microorganisms such as fungal or bacterial can better use this assimilated carbon for plant growth (Soares and Rousk, 2019). Well-grown plants usually have more root exudates, which play an important role in changing the organic or inorganic matter in the soil and are also a continuous source of organic matter (Wang et al., 2017). Moreover, it is also well reported that rice root will slowly die after the grain filling (decreased root length, root tip numbers, etc.); and these root residues will remain in the soil as an additional supplement to the carbon source (Hongyan et al., 2018).

3.2. Rice biomass, yield and its components

The biomass of rice root, stem and leaf remarkably increased under the concentrations of 20 and 50 μM H₂O₂, when compared with CK (Fig. 2). And the increment of root, stem and leaf biomass under the treatment of H₂O₂ of 20 μM were most significant, increased by 31.97%, 46.28% and 38.65% respectively, but there was no significant difference under the treatment of 100 μM H₂O₂ compared with CK ($p > 0.05$). Therefore, it was demonstrated from our results that simulation of natural precipitation in the micromolar concentration of H₂O₂ can increase the rice biomass, but with the increased concentration H₂O₂ at micromolar level, the biomass of rice decreased contrarily.

Similarly, rice yield also increased under H₂O₂ treatments, and the theoretical value of rice yield increased most significantly ($p < 0.05$) under 20 μM H₂O₂, increasing by 38.08%. And the decisive component factor, the effective panicles, increased by 11.49% under the treatment of H₂O₂ of 20 μM (Table 1). In addition, the grain number per panicle, the setting rate were also highest under 20 μM H₂O₂. However, there was no significant difference of the thousand grain weight of rice under different H₂O₂ concentrations ($p > 0.05$). Thus, low H₂O₂ concentration could remarkably increase the rice output. These findings were probably due to the optimization of soil nutrient status and soil condition, including the increment of the pH value (Fig. 1), and more N, P, K nutrients released from the degraded pig manure (Lai et al., 2008; Sholly et al., 2010; Méndez et al., 2012).

3.3. Cu and Zn content in different parts of rice

The Cu and Zn content in rice roots dropped with H₂O₂ application compared with CK, and the lowest Cu and Zn content were found in 20 μM, decreased by 44.62% and 36.91% respectively (Table 2). However, Cu content in stem, leaves and grain showed no significance at 20 μM H₂O₂, compared with CK. Contrarily, 50 and 100 μM H₂O₂ increased Cu in grain, this might due to the increased Cu transport efficiency from straw to grain in these treatments. As the translocation factors were 0.72, 0.72, 1.39 and 0.83 for 0, 20, 50, 100 μM H₂O₂ treatments respectively. And Zn content in rice roots showed similar pattern with Cu (Table 2). Zn content in steam and leaves do not have obvious difference, but the H₂O₂ treatment markedly decreased the Zn

Table 1
Effects of H₂O₂ concentrations on rice yield and its components.

H ₂ O ₂ concentrations (μM)	0	20	50	100
1000-grain weight (g)	19.83 \pm 0.30a	19.85 \pm 0.22a	20.31 \pm 0.55a	19.96 \pm 0.43a
Effective panicles ($\times 10^4$ /hectare)	367.3 \pm 11.81b	415.0 \pm 6.80a	408.2 \pm 11.78a	326.5 \pm 11.78c
Grain number per panicle	95.4 \pm 0.23c	136.5 \pm 4.46a	118.3 \pm 4.74b	107.1 \pm 3.28bc
Setting rate (%)	58.45 \pm 1.52c	80.00 \pm 4.02a	70.89 \pm 0.79b	66.65 \pm 2.52bc
Ideal yield (t/hectare)	6.97 \pm 0.34c	11.24 \pm 0.32a	9.78 \pm 0.25b	6.99 \pm 0.44c

All values are presented as mean \pm standard error (n = 4) and bars with different letters indicate significant difference among H₂O₂ concentrations ($p < 0.05$).

Table 2
H₂O₂ effects on the Cu and Zn content in different parts of rice (mg/kg).

Tissues	Cu				Zn			
	0 μ M	20 μ M	50 μ M	100 μ M	0 μ M	20 μ M	50 μ M	100 μ M
Root	20.4 \pm 0.51 a	11.3 \pm 0.25 c	12.9 \pm 0.32 bc	14.4 \pm 0.79 b	85.1 \pm 5.79 a	53.7 \pm 2.84 b	57.7 \pm 0.82 b	74.2 \pm 3.63 a
Stem	4.6 \pm 0.38 a	3.4 \pm 0.17 a	3.5 \pm 0.03 a	3.8 \pm 0.36 a	21.3 \pm 1.96 a	15.7 \pm 1.98 a	20.7 \pm 2.08 a	19.0 \pm 1.18 a
Leaves	4.8 \pm 0.08 b	5.2 \pm 0.36 b	5.0 \pm 0.15 b	6.7 \pm 0.06 a	25.0 \pm 0.29 a	28.6 \pm 1.40 a	26.7 \pm 1.45 a	25.9 \pm 0.84 a
Grain	6.4 \pm 0.73 b	6.2 \pm 0.87 b	10.47 \pm 0.67 a	8.8 \pm 0.48 a	34.4 \pm 2.34 a	23.2 \pm 1.48 b	24.5 \pm 1.5 b	27.5 \pm 2.24 b

All values are presented as mean \pm standard error (n = 4) and bars with different letters indicate significant difference among H₂O₂ concentrations ($p < 0.05$).

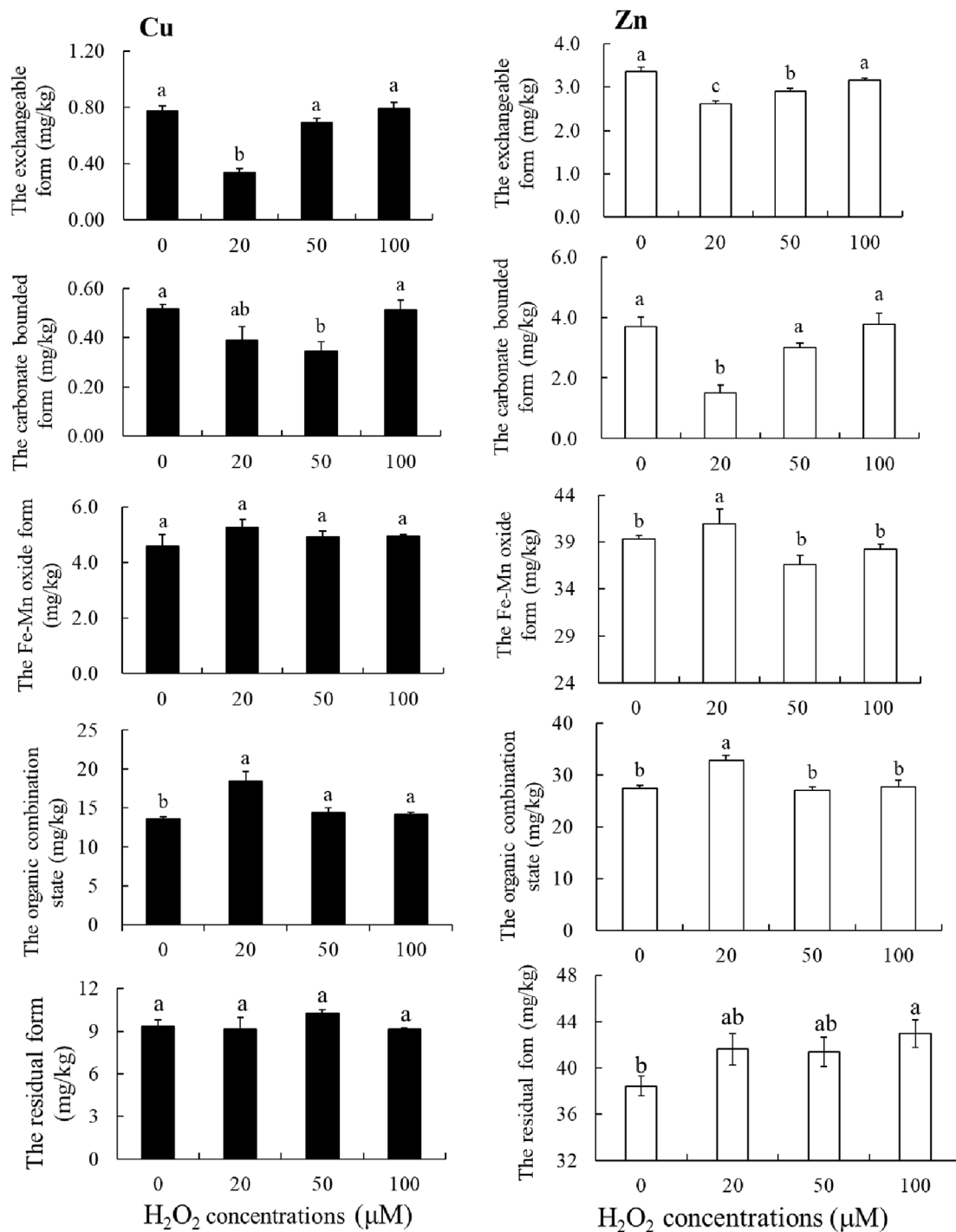


Fig. 3. H₂O₂ effects on the Cu and Zn morphological change in soil. All values are presented as mean \pm standard error (n = 4) and bars with different letters indicate significantly different significant difference among H₂O₂ concentrations ($p < 0.05$).

content in rice grain. And 20 μM H_2O_2 had the lowest Zn content in grain, decreased by 32.49% comparing with CK. According to the Chinese Food Hygiene Standard (NY861-2004), both Cu and Zn content in grain did not exceed the standard (except Cu content in 50 μM). But with micromolar concentration of H_2O_2 at a low concentration, especially 20 μM H_2O_2 , it was conducive to alleviate Cu and Zn stress to rice. These results indicated that effects of rain-borne H_2O_2 on manure derived Cu and Zn were different, though it had good potential in lowering the Cu and Zn content in rice organs, especially in rice root.

3.4. Cu and Zn morphological change in soil

The chemical speciation forms of heavy metals, rather than the total heavy metal concentration, are the key factors for determining the mobility, toxicity, and bioavailability of these metals (Liu et al., 2007). Though the total Cu and Zn content were higher with exogenous H_2O_2 application, their morphological change patterns were different. Additionally, the inactive components (carbonate bounded form, Fe–Mn oxide form and residual form) accounted for the majority of the total Cu and Zn content, even as high as 97.8% (Cu) and 96.6% (Zn) in 20 μM treatment. Compared with CK, the Cu concentration in exchangeable form and carbonate bounded form dramatically decreased by 56.03% and 24.52% in 20 μM treatment. Contrarily, the organic combination state, a more stable form, increased significantly in 20 μM treatment, increased by 26.50%. And there was no significant difference in other Cu form, such as Fe–Mn oxide form and residual form ($p > 0.05$), though Cu content in Fe–Mn oxide form under the 20 μM treatment increased by 12.95% (Fig. 3). The concentrations of Zn in different forms were similar to those of Cu. The concentrations of Zn in exchangeable form and carbonate bounded form decreased under different H_2O_2 treatments compared with CK. And Zn content in exchangeable form and carbonate bounded form decreased most significantly at 20 μM H_2O_2 , decreased by 21.89% and 59.28% respectively. The concentration of Zn in Fe–Mn oxide form and organic combination state under 20 μM , on the contrary, increased notably, by 9.60% and 8.44% respectively. However, there was no significant difference in other H_2O_2 levels ($p > 0.05$). Thus low H_2O_2 concentration could noticeably reduce the concentration of Cu and Zn in exchangeable form and carbonate bounded form in soil, but remarkably increase Fe–Mn oxide form and organic combination state.

It has been documented that H_2O_2 react with Fe^{2+} in soil to form Fe (OH)₃ colloids at a low concentration, which can reduce the activity and mobility of heavy metals by the action of adsorption and precipitation to adsorb and precipitate heavy metals (Bolan et al., 2013). And the generated hydroxyl radical in the Fenton reaction is highly oxidizing to many substances, such as a wide number of organic molecules or other heavy metals (As, Cd, etc.) (Lee and von Gunten, 2010). Additionally, Fe(OH)₃ can improve soil pH value for its alkalinity, and drop the negative charge of soil surface, thus increase the heavy metal adsorption to alleviate toxic effects on crops (Jackson and Punshon, 2015). Noticeably, the migration and transformation of heavy metals in soil is a dynamic process. The physical composition and chemical properties of soils directly affect the existing forms of heavy metals. And soil pH was found to play the most important role in determining, metal speciation, solubility from mineral surfaces, movement, and eventual bioavailability of metals (Zhao et al., 2010; Zeng et al., 2011). Apart from soil pH, organic matter in soil is also one of the most important soil properties affecting heavy metal availability (Amir et al., 2005; Park et al., 2013; Tang et al., 2014). Thus from our results in Fig. 1, H_2O_2 can both significantly increase soil pH and the organic matter content, which indirectly affect the Cu and Zn morphological changes in soil. In addition, low H_2O_2 application at micromolar level decreased Cu and Zn contents in the exchangeable form and carbonate bounded form (Fig. 3), which probably contributed to a better rice growth and rice yield, and lower Cu, Zn accumulation in rice (Table 2).

4. Conclusion

These results obtained from this study shed some light on the potential role of rainwater-borne H_2O_2 in changing manure-derived Cu and Zn speciation and accumulation. Micromolar concentration of H_2O_2 at a low concentration, may react like Fenton process, showed advantageous properties on this rice-soil system. The exogenous application of H_2O_2 increased the soil pH and organic matter content, the pH and organic matter content were both increased most significantly under the treatment of 20 μM H_2O_2 . Biomass of rice root, stem and leaf remarkably increased with the application of 20 and 50 μM H_2O_2 . Meanwhile, the ideal rice yield, setting rate, grain number per panicle were all increased at 20 μM H_2O_2 . Cu and Zn content in exchangeable form and carbonate bounded form dramatically decreased in soil, and contrarily the organic combination state increased significantly in 20 μM treatment. Thus Cu and Zn accumulation in rice decreased, especially in rice root. Applying low concentration H_2O_2 decreased Zn concentration in rice grain, but showed no significant effects on Cu accumulation in rice grain. In summary, 20 μM H_2O_2 indicated the best effects on easing the Cu and Zn toxicity derived from dry-fermentation-bed pig manure, but further study is still needed to explore the interaction mechanisms among micromolar concentration of H_2O_2 , pig manure and rice-soil system.

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我国与欧美化肥重金属限量标准的比较和启示

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摘要: 本文介绍了欧盟各国以及美国部分州的化肥重金属限量标准的制定过程和阈值, 分析了我国相关标准制定中存在的不足。我国化肥重金属限量标准是针对肥料种类来制定的, 肥料种类之间重金属限量值及限制的重金属种类均存在很大差异。限量标准有国家标准, 也有行业标准。不少单质化肥及复合肥料标准中没有重金属限量指标, 但对由单质化肥配制的水溶性复混肥料则制定了非常严格的限量标准。我国重金属限量标准的制定缺乏理论依据和试验数据支撑, 标准公布后也缺少制定依据的说明和解释。美国是根据磷肥及微量元素肥料种类和用量两方面来设定重金属限量标准, 该标准由美国环保局 (USEPA) 等机构根据化肥中有害元素对人类健康风险评估模型制定的, 并参考了长期的田间定位试验数据和食物中重金属的风险评估调查数据。美国植物食品控制管理协会 (AAPFCO) 根据上述风险评估结果提出了化肥中重金属的限量标准, 美国各州则在参考该标准的基础上根据各州法律法规实施。欧盟目前没有统一的化肥重金属限量标准, 各国各自规定了相关标准。建议我国参考发达国家的标准制定方式建立重金属的危害风险评价体系; 根据大量、中量与微量元素肥料类别及施用量, 区分不同重金属的限量, 且所有化肥所含的重金属进入农田的限量标准应保持一致。建议制定标准应进行环境、食品安全等调查与长期定位试验, 以科学数据为依据, 制定适合我国国情的化肥重金属限量标准。

关键词: 化肥; 重金属; 限量标准

Comparison of heavy metal limits for chemical fertilizers in China, EU and US and enlightenments

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Abstract: This paper briefly introduced the work out procession and the heavy metal limits in chemical fertilizers in some states of the United States and the Europe Union countries, and pointed out the shortages existed in the standards and the working out processions in China. The heavy metal limit standards for chemical fertilizers in China are fertilizer type specific, and greatly different among the fertilizers. The heavy metal limit standards are issued by different departments, including national and industrial standards. Some of the national standards for simple fertilizers and compound fertilizers lack heavy metal limits, but that for water-soluble fertilizers has quite strict limits for heavy metals. The limit standards for heavy metals do not have adequate theoretical and experimental data support, and have no necessary explanations for the basis of establishment after publication. The heavy metal limits in the United States are set up considering both the phosphate and micronutrient fertilizers and their application amounts. Several institutions (e.g. USEPA) in the United State made the standards using the evaluation models for the risks assessment of harmful elements in fertilizers for human health, and referring the data from long-term local field trials and the surveys for risk assessments in foods. The Association of American Plant Food Control Officials (AAPFCO) has proposed limits for heavy metals in chemical fertilizers based on

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these acceptable limits, and some states in the US are implementing the heavy metal standards based on their state laws and regulations. The EU countries do not have uniform heavy metal limits in chemical fertilizers, and they manage the fertilizers by their own laws and regulations. The standard settings for heavy metal limits relate to various benefits and public welfare issues in fertilizer production and application, soil health, water environmental protection, food safety, deserving our careful thinking and investigation. Suggestion: China should refer to the standard setting methods in developed countries to establish assessment system for hazard risk of heavy metals. The heavy metal limits should consider fertilizer types such as macronutrient, secondary and micronutrient fertilizers, and make one standard for all chemical fertilizers in China. Meanwhile, by referring to the existing research results from foreign countries, it is better to jointly formulate heavy metal limit standards for chemical fertilizers suitable for our national conditions by various departments based on investigation and long-term fixed experiment.

Key words: chemical fertilizers; heavy metals; limit standards

根据我国 2014 年国家环境保护部和国土资源部发布的《全国土壤污染状况调查公报》显示, 土壤重金属镉、汞、砷、铜、铅、铬、锌、镍 8 种重金属元素的点位超标率分别为 7.0%、1.6%、2.7%、2.1%、1.5%、1.1%、0.9%、4.8%。工矿业、农业生产等人类活动和自然背景值高是造成土壤重金属超标的主要原因, 部分地区土壤污染严重^[1]。土壤重金属污染来源很多, 如大气沉降、污水灌溉、污泥施用、有机肥和化肥的施用等。化肥在提高农作物产量的同时, 也会带入有害的重金属元素^[2]。由于化肥生产原料和工艺的不同, 不同化肥产品重金属的含量差异很大, 磷肥的生产原料是磷矿石, 由磷矿石原料带入磷肥中的重金属元素含量较高, 而钾肥和氮肥中重金属的含量相对较低^[3-5]。中国作为农业大国, 是世界上最大的肥料生产国和消费国。据国家统计局统计, 我国 2016 年农用化肥用量达 5984.1 万 t (折纯量)^[6], 如果化肥中重金属含量限制不严格, 长期施用重金属含量高的化肥会造成土壤重金属的累积^[7]。因此, 为防止化肥中重金属在土壤的累积, 很多国家都制定了重金属的限量标准。但是不同国家重金属限量标准存在很大差异。本文综述了我国现行的化肥重金属限量标准, 并与美国、欧盟的化肥重金属限量标准进行了比较, 旨在为我国相关部门对现有的化肥重金属限量标准的修改或新的限量标准制定提供参考。

1 我国化肥重金属限量标准与存在的问题

为了规范化肥的生产, 防止重金属在土壤中累积, 我国制定了部分化肥中重金属的限量标准。我国肥料及水溶肥料标准规定了 5 种有毒重金属砷、镉、铅、铬、汞的指标要求; 而其他一些专用肥料,

如硫酸铜、微量元素叶面肥料、含氨基酸叶面肥料只规定了砷、镉、铅的限量标准; 硫酸铵规定了砷和铅的限量标准; 氯化铵只对铅的含量进行了规定 (表 1)^[8-18]。由我国部分肥料的产品标准 (国标、国家或行业推荐标准) 可知, 不同肥料产品的重金属标准 (数量或种类) 差异很大。其中硫酸铵与工业磷酸对砷的要求非常严格, 分别为 0.5 与 1 mg/kg。硫酸铵中铅的限量标准值是氯化铵的 10 倍, 除此之外, 我国肥料中砷、铅与铬的生态指标分别是水溶肥料限量标准值的 5 倍、4 倍与 10 倍, 而微量元素叶面肥料中砷、镉、铅则均是水溶肥料限量标准值的 2 倍, 由此可见水溶性肥料对有害元素的限制更为严格。

我国对大部分的单质肥料及复合肥都制定了国家标准, 具体有: 1) 氮肥, 尿素 (GB 2440—2001)、硝酸铵 (GB 2945—1989)、农业用碳酸氢铵 (GB 3559—2001)^[19-21]; 2) 磷肥, 钙镁磷肥 (GB 20412—2006)、过磷酸钙 (GB 20413—2006)、重过磷酸钙 (GB 21634—2008)^[22-24]; 3) 钾肥, 氯化钾 (GB 6549—2011)、农业用硫酸钾 (GB 20406—2006)、硫酸钾镁肥 (GB/T 20937—2007)^[25-27]; 4) 其它肥料, 如磷酸一铵、磷酸二铵 (GB/T 2025—2009)、农业用硝酸钾 (GB/T 20784—2013)、复混肥料 (复合肥料) (GB/T 15063—2009)、掺混肥料 (BB 肥) (GB 21633—2008)、缓释肥料 (GB/T 23348—2009) 等^[28-32]。这些肥料是市场销售的主流肥料, 在上述肥料的标准中, 均未对肥料中的重金属含量进行限定。

上述化肥的重金属限量标准为何有着如此明显的差别 (表 1), 这值得我们思考。一方面, 在已制定重金属限量标准的化肥中, 为什么重金属的数量与种类有如此大的差异, 其依据是什么? 另一方面, 上述化肥产品 (单质肥料或复合肥) 很多是水溶肥料

表 1 我国化肥与部分肥料原料的重金属限量标准 (mg/kg)
Table 1 The heavy metal standards of inorganic fertilizer and some fertilizer material in China

标准名称 Standard	As	Cd	Pb	Cr	Hg
GB 437-2009 硫酸铜 (农用) Copper sulfate (for crops use)	≤ 25	≤ 25	≤ 125		
GN 535-1995 ^a 硫酸铵 Ammonium sulphate	≤ 0.5		≤ 50		
GB/T 2946-2008 ^b 氯化铵 Ammonium chloride			≤ 5		
HG/T 4133-2010A 工业磷酸二氢铵 Ammonium dihydrogen phosphate for industry	≤ 50				
GB/T 2091-2008 ^c 工业磷酸 Phosphoric acid for industrial use	≤ 1		≤ 10		
HG/T 4511-2013 ^d 工业磷酸二氢钾 Monopotassium phosphate	≤ 50		≤ 50		
HG/T 2326-2015 工业硫酸锌 Zinc sulfate for industrial use		≤ 10	≤ 10	≤ 5	
GB/T 17420-1998 微量元素叶面肥料 Foliar microelement fertilizer	≤ 20	≤ 20	≤ 100		
GB/T 17419-1998 含氨基酸叶面肥料 Foliar fertilizer with amino acid	≤ 20	≤ 20	≤ 100		
NY 1110-2006 水溶肥料汞、砷、镉、铅、铬的限量及其含量测定 Limits and testing methods for Hg, As, Cd, Pb and Cr contents in water-soluble fertilizers	≤ 10	≤ 10	≤ 50	≤ 50	≤ 5
GB 23349-2009 肥料中砷、镉、铅、铬、汞生态指标 Ecological index of As, Cd, Pb, Cr and Hg in fertilizers	≤ 50	≤ 10	≤ 200	≤ 500	≤ 5

注 (Note) : a—硫酸铵 (GB 535-1995) 中的重金属限量标准为优等品的标准, 一等品与合格品不作重金属限量的要求; b—氯化铵 (GB/T 2946-2008) 的重金属限量标准为优等品的标准; c—工业磷酸 (GB/T 2091-2008) 的重金属限量标准为优等品的标准; d—工业磷酸二氢钾 (HG/T 4511-2013) 的重金属限量标准为优等品的标准。a—Ammonium sulphate (GB 535-1995) represents the heavy metal standards for the superior product, and there are no heavy metal limits for first grade and qualified products; b—Ammonium chloride (GB/T 2946-2008) represents the heavy metal limits for the superior product; c—Phosphoric acid for industrial use (GB/T 2091-2008) represents the heavy metal limits for the superior product; d—Monopotassium phosphate (HG/T 4511-2013) represents the heavy metal limits for the superior product.

(兑水施或通过灌溉系统施用的一类肥料) 的基础原料。如果水溶肥基础原料的限量标准不明确或限制不严格, 就容易造成水溶肥料中重金属含量超标。因此, 为何水溶肥料对汞、砷、镉、铅、铬的限量标准如此严格, 而不少单质肥料、复合肥料对重金属含量却不作任何限制, 其依据又是什么? 根据研究, 水溶肥料中的重金属主要来自生产原料, 且原料中的重金属一半以上来自磷肥和微量元素肥料。而我国水溶肥料重金属超标主要集中在砷和镉, 大量元素水溶肥中砷含量最高, 可达 61.0 mg/kg; 微量元素水溶肥以镉含量最高, 平均可达 3.90 mg/kg^[2, 33-34]。氮肥和钾肥中重金属含量极低, 作为水溶肥料的原料比较安全, 而磷肥 (磷酸二氢钾、磷酸一铵等) 和微量元素肥料中重金属含量普遍较高, 如作为生产原料, 需严格检验, 否则容易导致产品中重金属超标^[35-36]。

2 美国化肥重金属限量标准及其制定依据

2.1 美国部分州化肥重金属的限量标准

美国的肥料管理制度是由肥料法确定的, 目前

尚无联邦统一的肥料法, 而是各州根据当地的特点自主开展肥料立法, 各州在制定肥料法时均参考美国植物食品管理机构协会 (AAPFCO) 所提出的肥料法基本框架要求^[37-38]。在 AAPFCO 规定标准的基础上, 美国各州根据当地情况拟定各自肥料登记中化肥重金属的含量标准。其中, 华盛顿、加利福尼亚和俄勒冈州对化肥重金属规定了明确的限量要求^[39]。而有些州 (如密苏里) 在肥料登记时, 对重金属不做限量要求^[40]。

加利福尼亚州仅对化肥中的砷、镉与铅含量作了限量标准, 每 1% P₂O₅ 分别为 2、4 和 20 mg/kg; 每 1% 微量元素分别为 13、12 和 140 mg/kg^[41]。

在明尼苏达州所有肥料产品都必须登记。如果肥料厂家提供了重金属等非营养成分的数据, 该州农业部将对之进行评估。2003 年明尼苏达州议会通过了肥料中砷限量的法规 (MS CH 18C)。该法规规定肥料中砷含量不得超过 500 mg/kg^[41]。

华盛顿州的化肥重金属限量标准是按照华盛顿州法典—RCW15.54.800 所提的每年土壤所能接受的最大重金属添加量^[42]: As 336 g/hm²、Cd 88.5 g/hm²、Hg 21 g/hm²、Ni 807 g/hm²、Pb 2244 g/hm²、Se 61.5 g/hm²、

Zn 8295 g/hm²。该标准是根据 1996 年 8 月所公布的加拿大贸易备忘录 T-4-93 制定的。1979 年在《肥料法案和法规》的授权下,加拿大农业和农业食品局(AAFC)推出了一系列化肥的重金属限量标准。从 1993—1995 年期间,AAFC 重金属标准在其它地方的标准制定过程中进行了重新评估,结果发现 AAFC 重金属标准仍然有效^[43]。该标准是基于土壤长期(45 年)可以累积的重金属的最大接受量,华盛顿州则改为每年土壤所能接受的最大重金属含量。

在俄勒冈州所有肥料产品的登记申请中必须包括产品中所含砷、镉、铅、镍的分析数据,且每个化肥产品标签都要标示重金属的含量。在实验室分析表中,则需要提供实验室所用的重金属分析方法与最小的检测限度^[44]。其中,在该地区的化肥重金属限量标准如表 2 所示。

德克萨斯州的所有肥料在销售之前都必须进行登记^[45]。其对肥料的重金属要求为:As 41 mg/kg、Cd 39 mg/kg、Hg 17 mg/kg、Cu 1500 mg/kg、Pb 300

mg/kg、Se 100 mg/kg、Zn 2800 mg/kg。

2.2 制定依据

美国环保局 (USEPA)、加州食品与农业部 (CDFA) 以及美国肥料协会 (TIF) 根据风险模型对化肥中有害元素进行了人类健康风险评估^[46]。他们建立了肥料重金属安全浓度的广义风险模型(图 1),主要包括以下内容:根据不同作物与土壤确定施肥量,然后计算 50~100 年连续施肥后由肥料带入土壤的重金属浓度(Kd 范围)与植物吸收的重金属量(PUF 范围),评估人类食用这些农作物所摄入的重金属量,建立人类食物中可接受的重金属浓度范围,根据这些基础数据计算可接受的肥料重金属含量(RBC)主要。这三个部门的评估结果都表明,几乎在所有的情况下,肥料中的重金属浓度不会对施肥者、施肥者家庭成员或公众造成危险。其中,该模型中 100 年的数据来源于英国洛桑试验站的长期田间定位试验的监测结果,50 年的数据来源于美国哥

表 2 俄勒冈州化肥产品的重金属标准 (mg/kg 每 1% 养分)

Table 2 Oregon standards for heavy metals in chemical fertilizer products (mg/kg per 1% nutrient)

肥料种类 Chemical fertilizer	As	Cd	Pb	Hg	Ni
磷酸盐肥料 ^a Phosphate fertilizer	9	7.5	43	0.7	175
微量元素肥料 ^b Micronutrient fertilizer	76	61	340	4.5	1330
含磷酸盐与微量元素肥料 ^c Fertilizer containing phosphate and micronutrient	76	61	340	4.5	1330
其它肥料 ^d Others	54	45	258	4.2	1050

注 (Note): 1) 如果产品含有磷酸盐与一种微量元素,则该产品中的重金属含量不得超过上表中 (a) 或 (b) 所述的标准; 2) 如果产品含有磷酸盐与两种或两种以上的微量元素,该产品所含的重金属含量不能高于上表中 (a) 或 (c) 所述的标准; 3) 如果产品不含有磷酸盐与微量元素,该产品所含的重金属含量不得高于上表中 (d) 所述的标准。1) When the product has a guaranteed analysis of available phosphate (P₂O₅) and has a guaranteed analysis of one micronutrient, the product could not contain more of any metal that higher than the resulting values as calculated in (a) or (b) above; 2) When the product has a guaranteed analysis of available phosphate (P₂O₅) and has a guaranteed analysis of two or more micronutrients, the product could not contain more of any metal that higher than the resulting values as calculated in (a) or (c) above. 3) When the product has no guaranteed analysis of available phosphate (P₂O₅) and no guaranteed analysis of a micronutrient, the product could not contain any more than the resulting values as calculated in (d).

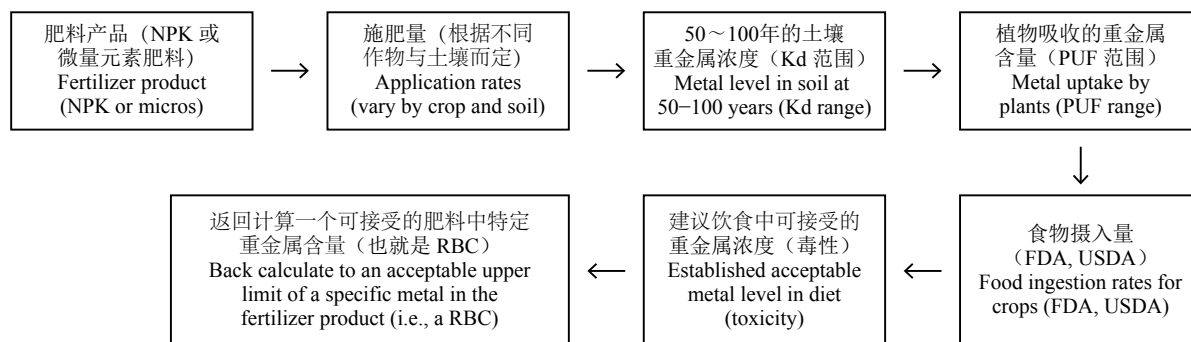


图 1 美国肥料重金属安全浓度的广义风险模型

Fig. 1 The generalized risk model for establishing safe levels of metals in America

伦比亚河流域灌溉项目的监测结果, 而 30 年的食物摄入重金属量来源于美国食品和药品管理局的“饮食研究”项目的调查监测数据。

根据该风险模型, 他们得到了可接受的风险浓度 (即 AAPFCO 的化肥重金属限量标准, 表 3)。表中列出了 9 种元素, 我国标准关注的 5 种元素都在其中。由于有害元素主要来自磷肥和微量元素肥料, 不同肥料配方添加的磷肥和微量元素肥料存在很大差异, 因此该标准以每 1% P_2O_5 和 1% 的微量元素为基本单位来设置限量标准。例如, 某一化肥产品若含有 10% 的 P_2O_5 , 则该肥料的重金属含量依次不得超过 130、100、1360、610、10、420、2500、260、4200 mg/kg (表 3)。

表 3 美国植物食品管理机构协会对化肥重金属限量的要求
Table 3 AAPFCO risk-based concentrations for inorganic fertilizers

元素 Element	大量元素肥料 NPK fertilizer (mg/kg per 1% P_2O_5)	微量元素肥料 Micronutrient fertilizer (mg/kg per 1% micronutrient)
As	13	112
Cd	10	82
Co	136	2228
Pb	61	463
Hg	1	6
Mo	42	300
Ni	250	1900
Se	26	180
Zn	420	2900

美国植物食品管理机构协会规定, 含有足量磷酸盐和/或微量营养元素的肥料, 其含有的金属含量超过上表所确定的金属含量时, 则被认为是掺假肥料 (不合格产品)^[39]。

3 欧盟化肥重金属限量标准及其制定依据

在 2003 年, 欧盟颁布了肥料法 (EC No. 2003/2003)。受该法规管制的肥料称为“欧盟肥料 (EC fertilizer)”, 欧盟肥料可以在欧盟市场内自由流通。不受欧盟肥料法管制的肥料称为“国家肥料 (National fertilizer)”, 这些肥料受各国的法律管辖。尽管在欧盟内的国家相互承认相关法规, 但具体的指标在欧盟内仍未统一。欧盟肥料法对单质化肥、复混肥、

无机液体肥、配方肥和微量元素肥等五大类肥料明确了养分含量指标。但没有规定这些肥料的重金属限量值。仅在该法规附录 III 中提及了高氮含量氮肥 (硝铵) 中铜的限定 (铜含量不得高于 10 mg/kg)^[47-48]。

在 2003 年之后, 各国对欧盟统一的肥料法 (EC No. 2003/2003) 陆续提出了多次修订方案, 在这些方案中也多次呼吁须限定化肥中所含重金属最大值^[49]。但是, 各国所制定的化肥重金属限量标准各不相同 (表 4)。例如, 对于磷肥中镉含量的限量值, 奥地利、芬兰与瑞典等国的标准分别是每千克 P_2O_5 镉含量为 75、22、44 mg。各国对于统一制定欧盟国家肥料中的重金属限量值, 还存在不少争议。在 2016 年的肥料法修订方案中提出了修改意见。该建议分别区分了大量、中量与微量元素肥料中的重金属限量标准。对于大量与中量元素肥料, 砷、铅、镉、镍、汞的限定标准分别为 60、150、60、2 和 2 mg/kg; 对于微量元素肥料, 砷、铅、镉、镍、汞的限定标准分别为 1000、600、200、2000 和 100 mg/kg (均以干物质的量来计算), 其中铬暂无限定^[50], 微量元素肥料为 B、Co、Cu、Fe、Mn、Mo、Zn 等单一或混合肥料。

这些标准限量值的提出建立在上述各种有害重金属元素对人类与环境健康风险评估结果的基础上, 同时也参考它们与土壤、饮用水、环境、人类健康相关的科学研究 (涉及联合国环境规划署、联合

表 4 欧盟部分国家化肥重金属限量标准与推荐标准
(mg/kg, DM)

Table 4 Maximum limit values for heavy metals in inorganic fertilizer of Europe Union countries

国家 Country	As	Pb	Cd	Cr	Ni	Hg
捷克 Czech	20	30	50	50	N/A	0.5
葡萄牙 Portugal	N/A	N/A	N/A	N/A	N/A	N/A
意大利 Italy	N/A	N/A	N/A	N/A	N/A	N/A
爱沙尼亚 Estonia	50	100	60	50	100	2
德国 Germany	40	150	60	N/A	80	1
奥地利 Austria	N/A	100	75	100	100	1
芬兰 Finland	25	100	22	300	100	1
瑞典 Sweden	25	100	44	300	100	1
希腊 Greece	N/A	N/A	60	N/A	N/A	N/A
法国 France	60	150	90	120	N/A	2
建议限量 Proposed limit	60	150	60	N/A	2	2

注 (Note): N/A—暂无限定 No limit values at present.

国欧洲经济委员会、世界卫生组织与各种研究报告等)。除此之外,该标准的提出还需广泛咨询欧盟各成员国与工业企业代表。但是,据 NIPERA 的研究,在后期评估过程中发现,在 197 个来自 12 个成员国的无机磷肥样品中,约 21% 的产品无法达到每千克 P_2O_5 镉含量 60 mg 的限量标准,但对于铅、砷、镍的标准都没有问题。因此,如果按照每千克 P_2O_5 镉含量 60 mg 的限量标准强制执行,很多无机磷肥产品会因为镉含量超标而无法在市场上流通。这些建议的标准限量值还处于讨论阶段,新的肥料法并没有公布实施,目前还是执行 2003 年颁布的《肥料法》。

4 分析与讨论

4.1 对不同化肥产品所适用的重金属限量标准不同的思考

由上述可得,我国不同肥料产品或肥料原料所适用的重金属限量标准不同,且在重金属标准(数量或种类)上的差异很大(表 1),这些肥料产品主要针对砷、镉、铅作了重金属限量要求,其制定依据值得我们思考。同时,不少市场流通的单质肥料及复合肥没有对重金属进行标准制定,而这些肥料不少是水溶性肥料的基础原料。这容易造成水溶肥基础原料的限量标准不明确或限制不严格而使得水溶肥料中重金属含量超标的问题。例如,肥料原料工业磷酸二氢铵(HG/T4133-2010)只要求砷 ≤ 50 mg/kg,对其它元素未作限量要求。而工业磷酸二氢铵是水溶肥料的常用原料,在水溶肥料配方中添加百分之几到百分之几十不等,也有不少厂家直接包装成水溶肥料商品销售。但如果工业磷酸二氢铵在水溶肥料配方中添加量超过 50%,根据砷 ≤ 50 mg/kg 的标准执行,水溶肥成品中砷含量容易超过 25 mg/kg,这是水溶肥料有害元素标准(NY 1110-2006)的 2.5 倍(表 1)。因此,为何水溶肥料汞、砷、镉、铅、铬的限量标准如此严格,而对不少单质肥料、复合肥料的重金属含量却不作任何限制,这个制定依据也值得探究。

我国不同肥料产品或肥料原料所适用的重金属限量标准不同,一方面,将出现各肥料行业竞争不公平的问题;另一方面,部分单质肥、复合肥重金属限量不明确或限制不严格也有可能对农业土壤环境造成一定的风险(特别是含磷肥料与微量元素肥料)。因此,科学评估化肥中的重金属风险,并明确这些制定的依据以保证肥料产业的健康发展非常重要。

4.2 对长期施肥导致的重金属累积与健康风险进行科学评估的必要性

我国于 1995 年颁布了《土壤环境质量标准》(GB 15618—1995),将土壤分为三级。其中,根据该土壤环境质量标准要求,为保障农业生产和维护人体健康,一般农田、蔬菜地等的土壤重金属含量应执行二级标准^[51-52]。在长期施用化肥的情况下,由化肥带入的重金属如何影响土壤环境质量是人们关注的问题。有研究表明在长期施用肥料的情况下,除镉以外,土壤中的重金属含量并不会发生显著的变化。王腾飞等^[53]以红壤稻田(始于 1981 年)和红壤旱地(始于 1991 年)长期定位施肥试验的土壤数据分析发现,长期施用化肥和稻草还田未见明显的重金属积累。王美^[54]分析测定黑土、潮土和红壤在 20 多年不同施肥措施下土壤和作物中重金属的含量,结果发现长期单施化肥对黑土、潮土、红壤中锌、镉含量没有显著影响。赵芸晨等^[55]通过 10 年的长期定点施肥试验发现,在年基肥量为磷酸二钾 300~330 kg/hm²、尿素 225~250 kg/hm²、硫酸锌 15 kg/hm²、硫酸钾 75~90 kg/hm²,追肥尿素共用 675 kg/hm²、磷酸二钾 330 kg/hm² 的情况下,土壤中铜、锌、锰、铁、铅与铬都没有超标,只有重金属镉严重超标(按《土壤环境质量标准》二级标准执行)。王改玲等^[56]在陕西合阳县 28 年的长期定位施肥试验发现,在 N 和 P_2O_5 施用量均为 337.5 kg/hm² 的情况下,施用氮、磷化肥对土壤中各种重金属的增加不明显。这些研究结果表明,长期施用化肥可能导致镉超标,但对其它有害元素则无明显影响,而镉主要来自磷肥。

由上述可知,我国也有不少关于长期施肥对土壤重金属累积影响的研究,是可以深入进行相关数据的整合分析,以开展化肥中重金属浓度的健康风险评估。根据美国、欧盟的化肥重金属限量标准可以得知,美国 AAPFCO 以 1% P_2O_5 为参考基准来限制大量元素肥料中的重金属含量;欧盟则以每千克干物质的大量/中量元素肥料为参考基准,其中镉的含量以 P_2O_5 的浓度为参考。这与上述长期定位试验中镉污染主要来自磷肥的结果有着很好的关联性,也说明长期定位试验的结果对于我们了解化肥中重金属对农田重金属污染与潜在风险有重要的指导意义,可见进行长期的化肥重金属风险评估非常必要。除此之外,肥料的施用一般都是以养分含量计算的,所以以 1% P_2O_5 为参考基准来限制化肥中的重金属含量也是有一定理论依据的。因此,根据长期

定位试验了解长期施肥导致的重金属累积,整合多方数据,继而进行科学风险评估是非常重要的,再在此基础上科学地进行标准的修订或制定。

5 结论与启示

1) 我国化肥中重金属的限量标准可以根据大量、中量与微量元素肥料等类别区分不同重金属的限量,所有化肥中重金属限量标准可以保持一个标准。

2) 应该考虑化肥中的养分含量来确定一些重金属的限量范围。

3) 可以借鉴发达国家进行的化肥有害元素对人类健康风险的评估方式,由评估报告得到可接受的化肥中重金属的浓度,科学评估我国化肥所含重金属对土壤、环境和人类健康的风险,并得到风险评估结果。同时,可以参考借鉴国外已有的研究成果,在调查与长期定位试验的基础上,以科学数据为依据,由多个部门联合制定适合我国国情的化肥重金属限量标准。

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农用聚磷酸铵在土壤中的有效性研究进展及在农业上的应用

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摘要: 近年来, 农用聚磷酸铵作为一种新型肥料逐渐进入我国化肥领域, 常用作高浓度液体复合肥料的基础磷肥。聚磷酸铵 pH 值近中性, 结晶温度低, 具有螯合性、缓释性, 有着很大发展空间。本文综述了聚磷酸铵在土壤中的有效性(溶解性与移动性)的影响因素, 重点分析水解速率、土壤矿物、土壤质地与水分对聚磷酸铵在土壤中的有效性, 并分析聚磷酸铵在农业上的应用与发展前景。

关键词: 聚磷酸铵; 土壤有效性; 农业应用

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聚磷酸铵是一种含氮和磷的聚磷酸盐, 简称 APP, 其通式为 $(\text{NH}_4)_{n+2}\text{P}_n\text{O}_{3n+1}$ 。按其聚合度大小, 可分为低聚、中聚和高聚 3 种。聚合度越高, 水溶性越小。通常, 当 $n < 20$ 为水溶性, $n > 20$ 为水不溶性, 而作为肥料用的聚磷酸铵聚合度通常为 $5 \sim 18$ ^[1-3]。APP 最早由美国田纳西流域管理局在 20 世纪向世人介绍^[4], 而我国聚磷酸铵的研制与生产起步于 20 世纪 80 年代^[5]。在国外, 液体聚磷酸铵肥料已得到广泛使用, 可用作配制高浓度液体复合肥料的基础磷肥。固体聚磷酸铵产品一般由 41% 正磷酸盐 (PO_4^{3-})、54% 焦磷酸盐 ($\text{P}_2\text{O}_7^{4-}$)、4% 三聚磷酸盐 ($\text{P}_3\text{O}_{10}^{5-}$) 与 1% 的四聚及四聚以上的多聚磷酸盐组成, 不同厂家生产的产品存在聚合度比例的差异。液体聚磷酸铵产品一般比颗粒产品含更高的三聚磷酸盐与正磷酸盐, 并且组分是变化的, 常见的范围是 30% ~ 40% 正磷酸盐, 50% ~ 55% 焦磷酸盐, 以及一部分三聚与四聚磷酸盐。国外常用液体 APP 配比有: 8-24-0、10-34-0、11-37-0、11-44-0、8-28-0 等, 固体为 12-57-0^[6-8]。利用上述液体 APP 与氮溶液、钾肥混合可生产液体混合肥。固体 APP 也可用于颗粒复合

肥的生产, 代替一部分磷铵。目前我国专业生产聚磷酸铵肥料的企业尚少, 其性状、组成及生产方法尚在研发阶段, 产量估计 3 万 t 左右^[3]。而美国现有 130 家工厂生产农用 APP, 年产量可达 200 万 t。与国外相比, 在产品质量、数量和应用方法上都存在较大的差距^[9]。作为新型磷肥, APP 在我国有着广阔的发展空间。

磷主要以扩散的方式从施肥点开始向四周移动, 因此土壤的吸附与沉淀反应会严重影响和限制磷的移动, 并影响植物对磷的吸收^[10]。如果磷在土壤中可以保持相对长久的溶解性与移动性, 则可以大大提高磷肥的利用率。本文将从水解速率、土壤矿物、土壤质地与水分等直接或间接影响聚磷酸铵有效性的因素进行综述, 以期为聚磷酸铵在我国的生产和应用提供理论依据。

1 聚磷酸铵在土壤中有有效性的影响因素

1.1 水解速率对聚磷酸铵有效性的影响

聚磷酸盐不能直接被植物吸收(少数研究表明焦磷酸盐可以直接被植物吸收), 只有水解为正磷酸盐后才能被植物吸收利用, 因此水解反应直接影响了植物吸收聚磷酸盐^[11-12]。当施入到土壤中后, 聚磷酸盐会逐渐水解为正磷酸盐; 聚磷酸盐在土壤中的水解速率决定着它们在土壤中移动的形式与距离, 从而影响作物对其的吸收。由于与土壤固相反应不同, 当添加到土壤中时, 聚磷酸盐离子可能比正磷酸盐离子有着更好的移动性^[7, 13-14]。但当聚磷酸盐离子水解后, 移动性则降低。因此, 聚磷酸盐的移动性很大程度取决于其水解速率。如果水解速

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率大于扩散速率,磷的移动主要以正磷酸盐的形式进行,这样聚磷酸盐的移动与正磷酸盐相差不大。如果水解速率缓慢,比如在强碱性或钙质土壤中(且微生物活性较低),磷主要以聚磷酸盐的形式移动,这时螯合反应将决定磷的扩散、磷的反应产物以及作物的磷吸收效率^[15]。

在酸性土壤中,Sample等^[16]的研究发现正磷酸铵与聚磷酸铵的施用没有显著的差异,可能是由于该试验中APP溶液50%的磷是正磷酸盐;同时,焦磷酸酶在土壤、微生物以及植物中广泛存在,很容易就使聚磷酸盐水解^[17-18]。APP一经与中性、酸性土壤接触,大部分聚磷酸盐在几天或几周内就被水解为正磷酸盐^[19]。Khasawneh等^[14]在砂壤土柱试验(pH值=6.0)中表施磷酸氢二铵(DAP)、焦磷酸三铵盐(TPP)、APP以观察它们的移动性,结果发现,DAP、TPP、APP的移动伴随着以下两种反应:(1)焦磷酸盐与聚磷酸盐逐渐水解为正磷酸盐;(2)磷酸盐阴离子与土壤的沉淀反应。该试验中,水溶性部分的聚合态磷水解很快,水解一半的时间为9~16 d。焦磷酸盐与聚磷酸盐的沉淀反应基本在1周就完成,并且是不可逆的,集中于一个明确区域;但正磷酸盐的沉淀反应却可以延续4周,沉淀速度随着时间与水溶性正磷酸盐浓度的增加而增加,且沉淀是分散的而不是聚集某一个点,并具有可逆性。在4周后,TPP、APP、DAP水不溶性磷的比例分别是58%、63%、45%。整体而言,聚磷酸铵水解速率的快慢,与土壤质地、酶活性、有机质含量、金属离子、pH值、温度等有关^[7 20-22],但随着粘粒含量和土壤可交换性铝、镁含量的增加,水解速率可能会有所下降^[23]。其中,焦磷酸盐的水解关键酶是磷酸酶,它可以快速催化APP水解,水解速率可比无酶催化快 10^6 倍^[18]。其它物理条件,如温度也是影响APP水解的重要因素,温度越高,聚磷酸盐水解越快。在15℃或低于15℃,水解速率很小。在27℃,会发生轻微水解;但是在30~40℃时,水解速度加快,并会在2~3个月内基本完成水解^[23]。pH值越低,APP水解得越快。在温度低于25℃,pH值近中性(pH值=6.4)时,APP是非常稳定的^[2 24-25]。

在钙质土壤中,不少田间和实验室研究结果发现,聚磷酸铵的肥效显著高于磷铵^[26-28],这可能是因为聚磷酸盐在碱性土壤中具有更好的稳定性。其中,焦磷酸盐、三聚磷酸盐较高的肥效可能与这

些聚磷酸盐螯合钙、镁的能力有关。由于焦磷酸钙(固相)的溶解度比磷酸钙大得多,因此焦磷酸盐离子持续存在时,可溶性磷酸钙盐变成不溶性磷酸钙盐的过程可能被抑制。Amer等^[29]的研究发现,当正磷酸盐与聚磷酸盐同时施入钙质土壤中时,聚磷酸盐离子可以使土壤溶液中正磷酸根的浓度更高。

1.2 土壤矿物对聚磷酸铵有效性的影响

聚磷酸盐与土壤矿物的反应速度、程度都与正磷酸盐不同。正磷酸盐与土壤矿物反应速度快,而聚磷酸铵相对较慢。正磷酸盐与土壤接触后几乎在数小时内即反应完成,但焦磷酸盐与矿物反应需要几天并持续几周,三聚磷酸盐则至少一周并可能持续数月^[13]。碳酸钙和碳酸镁与所有形态的磷酸盐反应都非常迅速。常温下,焦磷酸铵、三聚磷酸铵溶液与碳酸钙和碳酸镁反应迅速,但与黏土矿物、铁铝氧化物反应缓慢。黏土矿物的结构与组成会影响它们与聚磷酸盐的反应,黏土矿物所含有的少量其它矿物杂质也会影响反应产物。高岭石不受二聚或三聚磷酸盐的影响,蒙脱石会与之慢慢反应,硅镁石与之的反应会比较迅速^[30-32]。

聚磷酸盐与常用的正磷酸盐不同,它带有更多的磷原子,并有着更低的磷/氧比。这些特点给予聚磷酸铵更高的水溶性,且可以与金属离子(铁、铝、铜、锌等)形成稳定可溶的混合物。同时,这种混合物在土壤中可能保持更长时间的溶解状态。因此,磷酸盐沉淀之前,水溶性聚磷酸盐在土壤中可能比正磷酸盐移动得更远^[4 12]。Lindsay等^[33]的研究发现,在溶液中,焦磷酸盐可与钙、镁、铁、铝等螯合。将酸性砂壤土放在焦磷酸铵(pH值=6.25,含有50%磷)的饱和溶液中,摇动后,过滤酸性土壤中提取的聚磷酸盐时,发现没有沉淀产生,说明其生成了可溶性的聚磷酸铁及聚磷酸铝螯合物。用钙质粘壤土进行的相似试验表明,发现生成较少的铁和铝螯合物,但在过滤物中发现了 $\text{CaNH}_4\text{P}_2\text{O}_7 \cdot \text{H}_2\text{O}$ 沉淀。

中国地域辽阔,南北土壤类型各有不同,包括砖红壤、赤红壤、红壤、黄壤、黄棕壤、棕壤、黑钙土、栗钙土、棕钙土等^[34]。由于各土壤所含矿物质不同,它们对聚磷酸铵有效性的影响也不同。根据聚磷酸铵与土壤矿物反应的情况可知,在含铁、铝较多的土壤,聚磷酸铵有效性相对较高;在钙、镁含量高的土壤,其有效性则降低。虽然酸性

土壤的铁、铝含量高,碱性土壤的钙、镁含量高,但由于 pH 值的影响,聚磷酸铵在酸性土壤中的水解更容易发生。因此,聚磷酸铵在不同土壤类型的有效性表现很可能是土壤矿物质与水解速率综合影响的结果。

1.3 土壤质地与水分对聚磷酸铵有效性的影响

土壤中磷的移动性很低,扩散距离仅有 1 ~ 2 mm,且扩散速度很慢。而土壤养分只有到达根表后才可能被植物吸收。因此,提高磷的移动距离在一定程度上提高作物对磷的吸收^[35]。但大量研究表明,土壤质地的改变或水分的增加并不能显著改变聚磷酸铵的移动性。Lauer^[36]的研究发现,在不同土壤质地上,土表淋施聚磷酸铵时,其移动性较小,能向下移动距离约为 5 cm。其在砂土中移动最远,平均 6.2 cm。非钙质砂壤土次之(平均 5.1 cm),移动性最差的是钙质砂壤土,平均移动距离仅为 3.4 cm。增加灌溉量后(增加 3 倍),发现 APP 的移动不会出现大幅度的增加,在砂质土移动距离增加最大,为 25%;非钙质砂壤土增加了 18.6%;钙质砂壤土增加了 9.68%。Hashimoto 等^[7]用土柱表施磷肥的试验发现,在砂壤土上,所有测定的正磷酸盐(MAP 和 DAP)与聚磷酸盐的移动距离相似,约 4 ~ 5 cm,但水溶性磷含量最高值的所在位置略有不同。正磷酸盐的水溶性磷含量随着深度增加而逐渐减少,但聚磷酸盐的水溶性磷的最高值保留在距离表面 1 cm 处,且与聚磷酸盐的聚合度没有任何直接关系^[7,37]。但也有研究发现,施于土壤表面的聚磷酸铵液体肥料,有效磷向土壤迁移深度可达 15 cm^[38]。整体而言,聚磷酸铵中磷的移动也是有限的,不同质地土壤之间磷的移动无明显差别,而增加灌溉量也不能明显提高聚磷酸铵的移动。但灌溉量的增加会影响聚磷酸盐的水解,从而影响其有效性。研究表明,焦磷酸盐在有氧土壤系统中的水解随着土壤状况的改变而改变,水解一半的焦磷酸盐需要 4 ~ 100 d^[39-40]。当土壤淹水后,厌氧微生物的活性将大大提高。研究表明,淹水情况下可以提高聚磷酸盐的水解速率,其中液体 APP 比固体 APP 水解得更快。液体 APP 聚合态磷的半衰期值在厌氧条件下是 1.6 ~ 2.0 d;在有氧条件下是 5.2 ~ 8.7 d。固体 APP 聚合态磷的半衰期值在厌氧条件下是 3.9 ~ 9.2 d;在有氧条件下是 12.5 ~ 27.0 d^[41-42]。但也有研究表明,淹水或干旱情况下,聚磷酸盐的水解无差异^[43]。

2 聚磷酸铵在农业生产中的应用

固体化肥利用率一般为 30% 左右,而液体肥料可达 80%,利用率大大提升。在机械化施肥中,液体肥料发挥着更大优势^[3,44]。同时随着我国节水农业的发展与化肥农药零增长的趋势,农用聚磷酸铵作为高浓度液体复合肥料的基础磷肥去推广与应用,定位更为准确;也将为液体肥料的发展提供更为广阔的空间^[45]。目前,我国生产方式多以热法磷酸(磷酸铵)/尿素为主,产品的性状和组成还很不稳定^[46]。但已有少量的聚磷酸铵开始用于我国液体肥料的生产。聚磷酸铵液体肥在欧美等地早已普及,且是美国主流的磷肥品种^[3,10,45]。聚磷酸铵作为液体肥原料有两个重要优势:首先它比正磷酸盐水溶性更好,其次它可以在湿法生产过程中整合(在溶液中)大部分杂质,同时还可以加入一些微量元素^[47]。聚磷酸铵本身有着很好的溶解度,与微量元素反应可以保持更长的溶解状态^[4],且 pH 值近中性,缓冲性好,有很好的复配性^[2]。总体而言,农用聚磷酸铵在我国发展潜力巨大,近年内市场规模有望达到 100 万 t。但需要考虑温度和土壤 pH 值等因素对聚磷酸铵水解的影响,且国内受制于运输条件,产品的推广也存在一定难度^[45]。

农用聚磷酸铵在美国等发达国家常用于液体肥料生产(清液型与悬浮型),主要应用于大田,最常用且使用数量最多的是液体 APP: 11-37-0 与 10-34-0。田纳西流域管理局在 20 世纪 80 年代初开发了一套用聚磷酸盐代替正磷酸盐来生产悬浮肥料的工艺,在悬浮肥料中加入聚磷酸盐(P_2O_5 25% ~ 35%),改善了悬浮肥料的低温贮藏性能,并降低了生产成本^[48]。聚磷酸铵一方面可以用于种肥(启动肥),另一方面则用于作物整个生长期(追肥)。用于种肥时通常是施入犁沟或播种时侧施;用于追肥时可以条施、撒施,也可以灌溉施肥^[49]。在种肥上,常用的聚磷酸盐配比为 11-34-0,施肥点位于种子旁边 5.0 cm 与下方 5.0 cm 的位置(5 cm × 5 cm),可以促进苗期种子根系形成与出苗^[50]。

在田间应用上,农用聚磷酸铵表现出了较好的优势。Holloway 等^[51]研究发现,在澳大利亚石灰性土壤上,相同化学组分的液体 APP 的磷利用率是颗粒磷肥的 15 倍。Venugopalan 等^[52]通过对比重钙(TSP)、磷酸氢二铵(DAP)、硝酸磷肥(NP)、固

体 APP 和液体 APP 等肥料的小麦肥效试验,表明施用固体 APP 的小麦产量高于 DAP 和 NP。章守陶等^[53]以等养分含量的固体磷酸一铵 (MAP) 为对照,发现液体 APP 可使哈密瓜增产 3.0% ~ 8.4%。Holloway 等^[54]在小麦试验中发现液态 APP 效果优于其它颗粒固体肥料,第一年施用使小麦增产 14.0%,第二年残留的肥效使小麦增产 15.0%。但 Engelstad 等^[55]研究表明,在低温情况下,APP 的水解速度慢,很可能比正磷酸盐的有效性低。但也有研究表明,作为作物的氮源与磷源,APP 与正磷酸盐无明显差别^[49]。而最近的研究发现,在高 pH 值的钙质土壤上,将 MAP 与 APP 以一定比例 (80%:20%) 混合,可以使土壤中磷的有效性显著提高,从而减少磷的投入^[56]。而在本课题组的研究中,在玉米苗期盆栽试验中 (等养分的条件下),发现与工业级 MAP 相比,聚磷酸铵在酸性砖红壤上的肥效较差;而在碱性石灰性土壤中的肥效更好。

3 展望

随着节水农业与现代化农业的发展,水肥一体化技术将有巨大的发展空间。而水溶性肥料是水肥一体化技术的配套产品。氮肥和钾肥一般都是水溶的。单从溶解性来讲,磷肥原料是决定水溶性肥料质量的关键因素。聚磷酸铵具备优良的溶解性、缓释性与螯合性,是液体肥料生产中很好的磷源,也可以用于复合肥的磷原料,在我国化肥市场中有着巨大发展潜力。

目前,我国对聚磷酸铵在液体肥料中使用的研究还处于起步阶段。为了能让聚磷酸铵在我国农业生产中发挥更大作用,可以着手以下几方面的工作: (1) 吸收和借鉴发达国家在聚磷酸铵应用中已有的技术和经验,开展田间试验示范,验证聚磷酸铵在不同土壤类型、不同作物下的效果,并找出适用于田间生产的规律; (2) 结合我国国情和生产实践,开展有针对性的研究,包括聚磷酸铵配方、剂型、水解、移动等规律、机理的探索,以此根据不同作物、土壤作出相应的聚磷酸铵配方与施肥方案; (3) 结合我国现代农业生产需求,研发多种以聚磷酸铵为基础原料的功能肥料,如利用农用聚磷酸铵混配性能,搭配除草剂、杀虫剂等使用。同时加大技术培训和推广力度,以满足农业现代化的需求。

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Research progress on the availability of ammonium polyphosphate in soil and its application in agriculture

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Abstract: The agricultural ammonium polyphosphates gradually enter the field of chemical fertilizers in China as a new fertilizer in recent years, and are commonly used as a basic phosphate for high concentration liquid composite fertilizer. The pH of ammonium polyphosphate is near neutral, with good properties of low crystallization temperature, chelating, and slow release, which has great potential as the new basic phosphate. In this paper, the factors influencing the availability (solubility and mobility) of ammonium polyphosphate in soil were reviewed. The effects of hydrolysis rate, soil mineral, soil texture and moisture on the availability of ammonium polyphosphate in soil were mainly illustrated. And we also analyzed the application and development prospect of ammonium polyphosphate in agriculture.

Key words: ammonium polyphosphate; soil availability; agricultural application

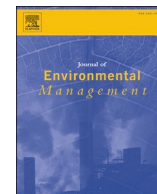
《中国土壤与肥料》征稿简则

《中国土壤与肥料》1964年创刊,是农业部主管、中国农业科学院农业资源与农业区划研究所和中国植物营养与肥料学会主办的全国性专业科技期刊。为全国中文核心期刊、中国科技核心期刊、中国农业核心期刊、RCCSE中国核心学术期刊。被中国科学引文数据库(CSCD)核心库、中国学术期刊综合评价数据库、CBST科学技术文献速报(日)、中国学术期刊文摘、CA化学文摘(美)、CABA农业与生物科学研究中心文摘(英)等收录。以促进土肥学科的发展为宗旨,加快成果转化、推动技术进步为目标。面向科研、教学和生产实践。主要刊登土壤资源与利用、植物营养与施肥、农业水资源利用、农业微生物、分析测试、环境保护、生态农业等方面的新理论、新技术、新产品的试验研究成果与动态。辟有专家论坛、专题综述、研究报告、分析方法、研究简报等栏目。读者对象为农业科研、教学、推广、环保及肥料生产、经营部门的科技、管理人员及农民技术员。

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1. 文稿请按“科技论文编写格式”撰写。要求论点明确、层次分明、数据可靠、图表清晰、文字精炼、标准确,有关数据进行统计分析。
2. 研究论文要有中、英文摘要和关键词。摘要中要含有论文的重要数据。
3. 量和单位及符号采用国家法定计量单位,符合国标对科技期刊的要求,不再使用N、M、ppm、rpm、亩、目等。土壤的磷、钾养分含量需用P、K计算,肥料的磷、钾养分含量用 P_2O_5 、 K_2O 计算。
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Research article

Co-application of earthworms and arbuscular mycorrhizal fungi enhances arsenic tolerance of upland rice and improves soil health

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ABSTRACT

Earthworms and arbuscular mycorrhizal fungi (AMF) are essential soil organisms that interactively shape soil-plant dynamics. This study elucidates the mechanistic basis of their co-inoculation in enhancing arsenic (As) tolerance in upland rice (*Oryza sativa* L.) and improving soil health in a pot experiment with As-contaminated soil (250.18 mg kg⁻¹). This study revealed that the inoculation effects of co-inoculation on rice biomass, N uptake, and P uptake were 86 %, 109 %, and 177 %, respectively, while reducing As concentration in shoot by 38 %. Physiological analyses revealed a 40.17 % reduction in malondialdehyde (MDA) content and a 6 % increase in superoxide dismutase (SOD) activity, indicating enhanced antioxidant capacity. Subcellular As compartmentalization shifted markedly, with organelle-bound As decreasing by 27 % (roots) and 48 % (leaves), while soluble fraction and cell wall sequestration increased. Soil health metrics improved, evidenced by elevated catalase (38 %), urease (15 %), and acid phosphatase (39 %) activities, alongside a 13 % reduction in bioavailable As fractions (As-F1 and As-F2) due to increased As-F4 stabilization. These findings demonstrate that earthworm-AMF synergy mitigates As toxicity by dual strategies: (1) enhancing plant antioxidant defenses and subcellular As compartmentalization, and (2) promoting plant growth via soil enzyme activation and nutrient cycling. This integrated approach offers a scalable, eco-sustainable strategy for safe rice cultivation in As-contaminated agroecosystems.

1. Introduction

Rapid industrialization and urbanization over the past decades have caused heavy metal contamination of soils. Industry, transportation, mining, agricultural fertilizers, and pesticides are the main sources of heavy metals in the environment and their release can cause damage to soil texture and stability (Han et al., 2002; Nogawa et al., 2017). Accumulation of heavy metals in soils not only negatively affects soil health and function, but also poses a threat to humans and other organisms through biomagnification in the food chain (Antoniadis et al., 2019; Khan et al., 2021). Soil surveys by the Chinese Ministry of Agriculture show that nearly 10 % of the study area is heavily contaminated with As and other heavy metals (Chen et al., 2008). Excessive As levels disrupt physiological, biochemical, and morphological processes in plants (Sharma et al., 2018). As toxicity inhibits seed germination, causes growth slowdown, leads to yield loss, reduces chlorophyll content and photosynthetic efficiency (Kumar et al., 2022). As interaction

with protein sulphhydryl groups causes irreparable cellular damage (Spagnoletti and Lavado, 2015). Rice is a major crop in Asian countries, and high intake of As in rice may have serious public health implications (Abedi and Mojiri, 2020). Water scarcity hinders the sustainability of agricultural systems in different parts of Asia, with rice cultivation consuming more than half of the irrigation water in agricultural production (Hu et al., 2015). Therefore, the cultivation of upland rice is considered an effective way to improve water use efficiency for food production (Chan et al., 2013). In addition, aerobic environments have a high soil redox potential, which allows As(III) to be oxidized to As(V). During the oxidation process, clay minerals and Fe-Mn oxides in the soil can adsorb the As(V) in the soil solution, thus limiting the movement of As (Kim et al., 2014).

In natural ecosystems, arbuscular mycorrhizal fungi (AMF) exist in a mutually beneficial symbiotic relationship with 80 % of terrestrial plants, a relationship that benefits both organisms as the host plant provides the fungus with photosynthetic products in exchange for

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mineral nutrients and water (Jiang et al., 2017; Riaz et al., 2021). It has been shown that AMF extraradical mycelia can expand root uptake and regulate the adsorption of heavy metals (Zhang et al., 2018). AMF chelate and sequester heavy metals through their fungal structure. Meanwhile, their secreted glomalin-related soil proteins (GRSP) also immobilize heavy metals. This is the first physical barrier for AMF to directly affect the entry of heavy metals into plants (Holatko et al., 2021). Growth retardation, reduction in chlorophyll biosynthesis, and decrease in water and mineral uptake are the different phototoxic effects induced by As in plants (Moreno-Jimenez et al., 2012). The mechanisms of AMF could aid in boosting the As tolerance level of the host plant (Smith et al., 2010; Wang et al., 2020) showed that AMF inoculation increased dry weight and P content of *Solanum nigrum* (Sharma et al., 2017). found that AMF colonization enhanced the activity of antioxidant enzymes of wheat while significantly reducing arsenic-induced oxidative stress.

In soil systems, there are often multiple organisms present at the same time, such as earthworms. Although they are not in an upper or lower trophic level relationship, earthworms may influence the formation of mycorrhizal symbioses through bioturbation and physiological metabolism (Paudel et al., 2016; Vasutova et al., 2019). Previous studies have shown that earthworms and AMF can regulate host growth performance and heavy metal uptake, and therefore earthworm-AMF interactions are considered to be a highly promising bioremediation aid (Mahohi and Raiesi, 2021; Santana et al., 2019; Wang et al., 2021) found that earthworms and AMF significantly improved soil quality parameters, including enzyme activities and nutrient availability. The co-incubation treatment increased the activities of urease, catalase, and acid phosphatase by 60 %, 40 %, and 20 % respectively compared with the uninoculated treatment. Meanwhile, earthworms play an important role in terrestrial ecosystems as “soil ecological engineers”, regulating the transport and transformation of heavy metals in the soil-plant system through feeding, metabolism, and excretion (Chai et al., 2020; Cheng et al., 2021; Nannoni et al., 2011). Although earthworms and AMF interact directly with the rhizosphere of the host plant, the interactions between these two important organisms are poorly understood. Further studies are needed to understand how their interactions regulate the soil and influence plant function.

This study aimed to determine the contribution of AMF and earthworms to the growth and antioxidant systems of rice in As-contaminated soil and evaluate their effects on soil quality. We also explored the As accumulation and transport patterns in rice and analyzed the proportion of subcellular distribution of As. We hypothesized that (i) AMF and earthworms enhance As detoxification in rice by promoting plant growth, enhancing antioxidant enzyme activity, and reducing As uptake, and (ii) the interactions of AMF and earthworms are beneficial in promoting soil health.

2. Materials and methods

2.1. Soil and organisms

The soil used in the experiment was from the surface soil (0–20 cm) of the farmland around the Dabao Mountain mining area in Shaoguan City, Guangdong Province, China. The physical and chemical characteristics of the soil were: soil pH 6.78, SOM (Soil organic matter) 18.21 g kg⁻¹, TN 1.275 g kg⁻¹, AN 112.81 mg kg⁻¹, TP 0.885 g kg⁻¹, AP 54.6 mg kg⁻¹, AK 41.45 mg kg⁻¹, As 250.18 mg kg⁻¹. The soil samples were dried naturally, passed through a 2-mm sieve, and then autoclaved at 121 °C for 2 h to kill the indigenous microorganisms. The earthworms used in the experiment were *Eisenia fetida*, purchased from the farmers' market. Before inoculation, adult earthworms (fresh weight 0.45 ± 0.05 g, body length 6.5 ± 0.5 cm) were selected for domestication. Then they were washed with deionized water several times and put into Petri dishes for 24 h to empty the intestinal contents. After that, they were washed again and prepared for use. Arbuscular mycorrhizal fungi

(*Funneliformis mosseae*) were provided by Guangxi University, China. In this experiment, a mixture containing plant root segments, fungal hyphae, mycorrhizal fungal spores, and potting sand was used as an inoculant after 3 months of expansion using maize (*Zea mays*) as the host plant. The test plant was upland rice. The variety was Hanyou 711, provided by the Guangdong Provincial Academy of Agricultural Sciences. The seeds were disinfected with 10 % H₂O₂ for 15–20 min, washed with deionized water, and then soaked for 16 h and put into the incubator. After the seeds germinated, rice seedlings with consistent growth and a height of 15 cm were selected for transplantation.

2.2. Experimental design

The experiment was a two-factor trial with earthworm and AMF treatments and was conducted by growing rice in pots on contaminated soil with an As concentration of 250.18 mg kg⁻¹. There were a total of 4 treatments with 3 replications per treatment. The treatments were as follows: 1) CK: Planting rice without adding AMF and earthworms, with 150 g of a sterile inoculum and 10 mL of an inoculum filtrate added; 2) E: Planting rice and adding earthworms, with 150 g of a sterile inoculum and 10 mL of an inoculum filtrate added; 3) AM: Planting rice and adding AMF; 4) EAM: Planting rice and adding earthworms and AMF. This was accomplished by first sterilizing the pots 3 times with 75 % alcohol, filling each pot with 3 kg of sterilized contaminated soil, and transplanting uniformly grown rice seedlings into the pots. For AMF treatment, 150 g of mycorrhizal agent was added to 2/3 of the pots, and for earthworm treatment, 15 adult earthworms of uniform size were added to the pots. The plastic pots used in the experiment measured 25 cm in upper diameter, 19 cm in lower diameter, and 17 cm in height, and were fitted with nylon netting at the bottom of the pots to prevent earthworms from escaping. All pots were placed completely randomly in the greenhouse of the ecological farm of South China Agricultural University. The average temperature of the greenhouse was 30 °C during the day and 20 °C at night, and the relative humidity was 65 %. The soil water content was maintained at 65 % during the growing period. Additionally, the Hoagland nutrient solution with low phosphorus was added weekly to maintain plant growth. All samples were harvested after 70 days of planting (Jointing stage of rice).

2.3. AMF colonization rate

Firstly, the roots were cleaned and cut into 1 cm segments, and 30 segments were selected for each treatment; then, the roots were stained with acidic magenta for 40 min, and then were rinsed with 5 % lactic acid solution for 2–3 times until the rinsing solution became clear; finally, certain lengths of stained samples were placed neatly on the slides, and then were covered with coverslips after dropping 1–2 drops of lactic acid glycerol, and the infiltration rate was determined by grid-crossing method under the condition of 100–400 × in a light microscope. Stained root segments were evenly placed in Petri dishes with grid lines; infected root segments were counted as infested root segments, and finally, the infestation rate was calculated based on the ratio of the number of infested root segments to the total number of observed root segments (Wang et al., 2020).

2.4. Plant growth and chlorophyll content

Plants were harvested by dividing them into shoot and root parts, cleaned with water, and placed in an oven at 105 °C for half an hour, and then baked in an oven at 75 °C for 72 h until reaching constant weight, and weighed. Chlorophyll content: Fresh leaves were homogenized with 95 % ethanol (1:50, w/v) at 4 °C for 5 min, and then centrifuged (3000 r min⁻¹, 4 °C) for 10 min. The absorbance of the supernatant was measured at 645 and 663 nm and the chlorophyll content was calculated (Jian et al., 2019).

2.5. N and P concentration of plants and soils

Grind the shoot and root finely and weigh 0.1 g in a digestion tube. Add 5 mL of concentrated sulfuric acid and mix thoroughly. Then heat to digest, and add 5–10 drops of hydrogen peroxide on the way to decompose the organic matter and carbon that were not destroyed by the H_2SO_4 . This treatment converts organic nitrogen and organic phosphorus to inorganic ammonium salts and phosphates so that N and P can be determined separately in the same digest. After digestion, Plant N was determined by Nessler's colorimetry, and Plant P was determined by molybdenum antimony colorimetry. Soil alkaline nitrogen was determined by alkaline diffusion method. Soil available phosphorus was determined by ammonium fluoride-hydrochloric acid leaching method (Bao, 2000).

2.6. MDA content and antioxidant enzymes

0.5 g of fresh leaves of the plant were taken in a pre-cooled mortar, 5 mL of 0.05 M phosphate buffer (pH = 7.0) was added, the mixture was ground and then centrifuged at 3000 r min^{-1} for 10 min and the supernatant was taken for the assay. MDA concentration as well as SOD, POD, and CAT enzyme activities was determined by using kits (Nanjing Jiancheng Bioengineering Institute, China). MDA concentrations were expressed as $\text{nmol g}^{-1} \text{FW}$, and SOD, POD, and CAT activity levels were expressed as $\text{U g}^{-1} \text{FW}$ (Wang et al., 2021).

2.7. As concentration of rice

Weigh 0.18 g of the pulverized plant samples and place it in a digestion tube. Add 10 mL of nitric acid for pre-digestion. Then, cover the tube and put it into the microwave digestion instrument for digestion. After digestion, drive off the acid until the remaining volume is 1–2 mL. Wash all the digestion solution into a volumetric flask, adjust the volume to the mark, filter it, and then determine the As content by fluorescence photometer.

2.8. Subcellular distribution of As

The As content in cell walls, organelles, and soluble fractions of leaves and roots was determined by differential centrifugation. Fresh plant samples were mixed (1:20, w/v) with an extraction solution (250 mM sucrose, 50 mM Tris-HCl, pH 7.5, 1 mM dithiothreitol). The mixture was ground in a pre-cooled mortar. All the components in the mortar were transferred to a centrifuge tube and centrifuged at 4°C and 1500 g for 15 min to precipitate the cell wall fraction. The supernatant was aspirated and centrifuged at 4°C and 12,000 g for 30 min to precipitate the organelle fraction. The supernatant was the soluble fraction. The centrifuged cell wall fraction and organelle fraction were dried at 80°C , and 10 mL of nitric acid was added for microwave digestion. For the soluble fraction, 5 mL of the sample was absorbed and 5 mL of nitric acid was added for digestion. The arsenic content in the samples was determined by fluorescence photometer (Pandey and Khare, 2024).

2.9. Soil As fraction

For the determination of soil As fractions including non-specifically sorbed (As-F1), specifically sorbed (As-F2), amorphous and poorly-crystalline hydrous oxides of Fe and Al (As-F3), well-crystallized hydrous oxides of Fe and Al (As-F4), and residual phases (As-F5), the method described by (Wang et al., 2023).

2.10. Soil enzymes analysis

Soil catalase was determined by potassium permanganate titration. Soil urease and soil acid phosphatase activities were determined by using kits (Nanjing Jiancheng Bioengineering Institute, China). Soil

catalase activity was expressed as $\text{mg g}^{-1} \text{H}_2\text{O}_2$ and soil urease and acid phosphatase activities were expressed as U g^{-1} .

2.11. Extraction and determination of GRSP

Extraction and determination of GRSP based on the method were presented by Wright and Upadhyaya (Sf and AuthorAnonymous, 1996) and improved with minor modifications. The extraction steps of easy-extractable GRSP (EE-GRSP) were as follows: weigh 1.00 g of soil samples and add 8 mL of 20 mM sodium citrate solution (pH = 7.0). Mixed them well, and sterilized for 30 min (121°C , 0.1 MPa), and then centrifuged at 5000 g for 20 min after cooling, and the supernatant was the EE-GRSP extract. The extraction procedure of total GRSP (T-GRSP) was as follows: weigh 1.00 g of soil samples and add 8 mL of 50 mM sodium citrate solution (pH = 8.0). Mixed them well, and then set to be sterilized for 60 min (121°C , 0.1 MPa), and then centrifuged at 6000 g for 20 min after cooling, and then the supernatant was obtained. After leaching the sample four times until the supernatant became colorless, mix the supernatants and centrifuge the mixture at 10,000 g for 3 min to obtain T-GRSP. After the supernatant was developed color with Caulem's Brilliant Blue, the absorption value was measured at 596 nm on a spectrophotometer with bovine serum protein as the standard sample, and then the content was calculated according to the standard curve.

2.12. Statistical analysis

All experimental data are expressed as means \pm standard errors of three replicates. The results of the experiment were processed by EXCEL software and the data were analyzed by univariate and multivariate statistics using SPSS 27.0, while the results were graphically analyzed using OriginPro 2024 (Kama et al., 2023). The correlation of each parameter was analyzed using Pearson correlation.

To evaluate the effect of arsenic stress on earthworms, the growth inhibition rate (GIR) of earthworms was introduced and calculated as follows:

$$\text{GIR} = \frac{W_2 - W_1}{W_1} \times 100\% \quad (1)$$

where W_1 and W_2 are the fresh weight (g) of earthworms before and after the inoculation experiment, respectively.

To evaluate the growth-promoting effects exerted by earthworms and AMF, the inoculation effect (IE) of biomass, N and P was introduced; to evaluate the As accumulation and translocation pattern of rice, the Bioconcentration Factor (BCF) and Translocation Factor (TF) were introduced. The formula was as follows:

$$\text{IE} = \frac{A_2 - A_1}{A_1} \times 100\% \quad (2)$$

$$\text{BCF}_{\text{Shoot}} = \frac{C_{\text{Shoot}}}{C_{\text{Soil}}} \quad (3)$$

$$\text{BCF}_{\text{Root}} = \frac{C_{\text{Root}}}{C_{\text{Soil}}} \quad (4)$$

$$\text{TF} = \frac{C_{\text{Shoot}}}{C_{\text{Root}}} \quad (5)$$

where A_1 and A_2 are plant biomass (g), N uptake and P uptake (mg) before and after inoculation, respectively. C_{Shoot} , C_{Root} and C_{Soil} represent plant shoot As concentration (mg kg^{-1}), root As concentration (mg kg^{-1}) and soil As concentration (mg kg^{-1}), respectively.

3. Results

3.1. Plant growth and nutrient dynamics

The mycorrhizal colonization rate was determined after the plants were harvested (Table S1), and no mycorrhizal infestation of the root was found in the CK and E treatments. The mycorrhizal colonization rates were 22 % and 17 % in the AM and EAM treatments, respectively, which did not reach a significant level ($P > 0.05$). The mortality rate of earthworms in E and EAM treatments was 18 % and 15 %, respectively, and the growth inhibition rate of earthworms was 29 % and 25 %, respectively (Table S1). The addition of AMF reduced the mortality rate and growth inhibition rate of earthworms slightly but not to a significant level ($P > 0.05$) (Fig. 1a). visualizes the growth of rice after 70 days of planting, the combined inoculation grows better than the individual inoculation. Two-way ANOVA showed that AMF significantly affected

shoot biomass, while earthworms and AMF had an interaction effect on root biomass (Table 1). The order of biomass magnitude in the shoot was $EAM > AM > E > CK$ (Fig. 1b), and both inoculation with earthworms and AMF significantly increased the shoot biomass of rice ($P < 0.05$). The root biomass was $AM > E > EAM > CK$, and combined inoculation also slightly increased but did not reach a significant level ($P > 0.05$). The inoculation effects of E, AM, and EAM treatments were 56 %, 82 %, and 86 %, respectively, indicating that inoculation with earthworms and AMF had an important role in the growth of rice under As stress.

For mineral nutrient N and P content in rice (Fig. 1c and d), it can be seen that the E and EAM treatments significantly increased the shoot N concentration, but the AM treatment significantly decreased it ($P < 0.05$), and the changes in the root N concentration were different from those in the shoot, with only the E treatment significantly increasing the N concentration ($P < 0.05$). In addition, the inoculation effect of N uptake was 73 %, 64 %, and 109 % for E, AM, and EAM, respectively;

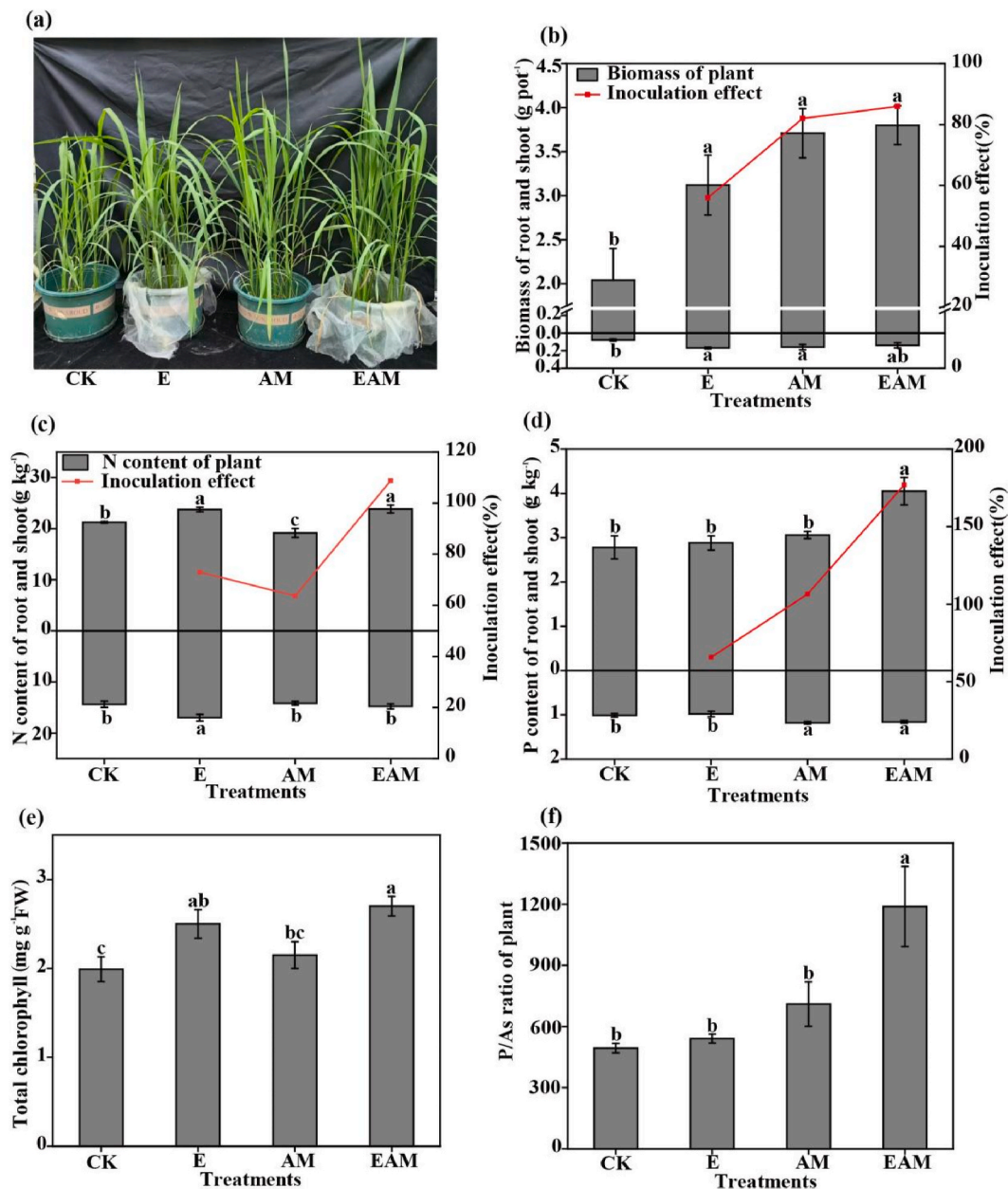


Fig. 1. Physiological indicators of growth in rice (a) rice growth, (b) rice biomass and its inoculation effect, (c) rice N concentration and N uptake inoculation effect, (d) rice P concentration and P uptake inoculation effect, (e) total chlorophyll content in rice, and (f) P/As of shoot. Data are presented in means \pm standard error. Different lowercase letters show statistically significant differences among treatments ($P < 0.05$).

Table 1

F-values and significances for the effects of earthworm (E) and AMF (AM) on each parameter by two-way ANOVA.

Parameters	E	AM	E*AM
Shoot biomass	3.678NS	14.61**	2.616NS
Root biomass	3.362NS	1.053NS	7.14*
Shoot P	6.156*	10.752*	4.13NS
Root P	0.3NS	18.972**	0.021NS
Shoot N	31.673***	2.464NS	2.968NS
Root N	9.177*	5.194NS	3.607NS
MDA	20.73**	33.907***	1.845NS
SOD	9.295*	0.92NS	0.035NS
POD	0.435NS	0.109NS	0.109NS
CAT	0.098NS	8.122*	0.779NS
Chlorophyll	14.058**	1.7NS	0.026NS
P/As	5.341NS	14.439**	3.616NS
pH	4.939NS	14.402**	1.351NS
SOM	22.05**	140.45***	31.25**
AN	96.996***	1.464NS	8.117*
AP	0.1NS	27.519**	0.409NS
Catalase	1.506NS	31.698***	0.88NS
Urease	15.868**	10.868*	0.679NS
Phosphatase	0.01NS	43.63***	0.11NS
EE-GRSP	1.923NS	5.622*	0.934NS
T-GRSP	0.135NS	9.348*	0.11NS
As-F1	0.105NS	115.019***	0.627NS
As-F2	0.042NS	10.698*	0.037NS
As-F3	2.298NS	13.147**	1.766NS
As-F4	1.777NS	1.863NS	0.083NS
As-F5	0.266NS	0.409NS	0.155NS
Shoot As concentration	2.464NS	12.126**	0.776NS
Root As concentration	0.043NS	11.528**	0.078NS
Shoot As accumulation	0.11NS	0.282NS	3.236NS
Root As accumulation	3.708NS	0.014NS	7.161*
BCFs	0.791NS	8.513*	0.51NS
BCFr	0.798NS	5.86*	0.006NS
TF	6.052*	4.225NS	1.609NS
Cell wall As of leaf	1.457NS	5.359*	0.537NS
Organelle As of leaf	0.124NS	31.72***	0.27NS
Soluble fraction of leaf	1.083NS	12.267**	0.085NS
Cell wall As of root	0.003NS	12.01**	0.158NS
Organelle As of root	0.511NS	9.265*	0.853NS
Soluble fraction As of root	0.19NS	1.098NS	0.977NS

* $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$; NS, no significance.

The order of magnitude of P concentration in the shoot was EAM > AM > E > CK, but only the EAM treatment reached a significant level ($P < 0.05$). In the root, P concentration was significantly higher in the EAM and AM treatments ($P < 0.05$), and the inoculation effect of P uptake was 66 %, 107 %, and 177 % in the E, AM, and EAM treatments, respectively.

It is noteworthy that the shoot P/As increased in rice after inoculation with both earthworms and AMF, were highest in the combined treatment (EAM), which was 2.4 times higher than that of the CK treatment (Fig. 1f). In terms of total plant chlorophyll, all the inoculated treatments were higher than the CK treatments (Fig. 1e), in the order of EAM > E > AM > CK, and both the EAM and E treatments reached a significant level ($P < 0.05$). Overall, the inoculation treatments promoted plant growth and the combined inoculation effect was greater than the individual inoculation effect.

3.2. Antioxidant response

Under As stress, ROS can be induced to accumulate in rice, causing damage to organelles and preventing normal plant growth. MDA is a bioindicator of lipid peroxidation in plants (Fig. 2a), shows the MDA content of different treatments, and two-factor ANOVA showed that earthworms and AMF significantly affected the MDA content ($P < 0.05$), but there was no interaction effect between them (Table 1). Individual inoculation with earthworms and AMF as well as combined inoculation reduced MDA content in rice ($P < 0.05$), and the greatest reduction was observed with combined inoculation. The MDA content was reduced by 22.89 %, 27.80 %, and 40.17 % in E, AM, and EAM treatments,

respectively. Further analyzing the antioxidant enzyme activities in the rice, different inoculation treatments showed different patterns (Fig. 2b, c, d), with earthworms significantly affecting SOD and AMF affecting CAT (Table 1). Compared to CK, E and EAM treatments increased SOD by 9.07 % and 6.62 %, respectively, while AM treatment decreased it by 3.63 %. For POD, there was no significant difference between them for either inoculated or uninoculated treatments ($P > 0.05$), indicating that bioinoculation had little effect on POD. It is worth noting the changes in CAT, which were reduced by all inoculated treatments compared to CK, by 5.88 %, 38.55 %, and 26.20 % for E, AM, and EAM, respectively, but only the AM treatment reached a significant level ($P < 0.05$).

3.3. Soil health and As speciation

Parameters in rhizosphere soil were measured to sort out the effects of sole and double inoculation of earthworms and AMF on soil micro-environment. The E treatment significantly reduced soil pH compared to CK ($P < 0.05$; Table 2) while other treatments were not significantly different. For SOM, it was reduced by 16.10 % in E treatment compared to CK, while AM and EAM treatments increased by 9.77 % and 11.17 %, with all inoculation treatments reaching significant levels ($P < 0.05$; Table 2). For soil mineral nutrients, two-way ANOVA showed that earthworms significantly affected alkaline nitrogen (AN) and there was an interaction effect between earthworms and AMF (Table 1), indicating that inoculation with AMF favored the promotion of AN by earthworms. AMF significantly increased available phosphorus (AP) ($P < 0.05$; Table 2). Almost all inoculation treatments increased AN and AP content. Compared with CK, the increase in AN was 112 %, 14 %, and 76 % for E, AM, and EAM, respectively; and the increase in AP was −1 %, 21 %, and 35 %, respectively, with earthworms contributing more to AN and AMF contributing more to AP. Soil enzyme activities are shown in (Fig. 3a, b, and c). Earthworms significantly affected urease and AMF significantly affected catalase and acid phosphatase (Table 1). AM and EAM treatments significantly increased soil catalase activity by 56 % and 38 %, respectively ($P < 0.05$). For soil urease, E, AM, and EAM were increased by 10 %, 8 %, and 15 % respectively. The soil acid phosphatase activity in descending order was EAM > AM > E > CK, and EAM and AM treatments reached significant levels ($P < 0.05$). Measurement of EE-GRSP and GRSP (Fig. 3d) revealed that AM treatment increased EE-GRSP content by 9 % compared to CK. As for T-GRSP content, AM treatment increased it by 4 %, and the size order of treatments was AM > EAM > E > CK, and the two-factor ANOVA showed that AMF significantly affected EE-GRSP and T-GRSP (Table 1).

The different fractions of As in the soil are shown in (Fig. 3e). The two-way ANOVA showed that AMF significantly affected As-F1, As-F2, and As-F3 (Table 1). Compared to CK, the E treatment showed little change in As-F1, As-F2, and As-F5, a 7 % increase in As-F3, and a 7 % decrease in As-F4; AM treatments were decreased by 16 %, 20 %, and 3 % in As-F1, As-F2, and As-F4, respectively, while As-F3 increased by 7 %; EAM treatments decreased As-F1, As-F2, and As-F4 by 13 %, 13 %, and 8 %, respectively, while As-F3 increased by 18 %. Overall, AM and EAM treatments contributed to the conversion of As-F1 and As-F2 to As-F3, resulting in reduced As bioavailability.

3.4. As uptake and its subcellular distribution in rice

Two-way ANOVA showed that AMF significantly affected shoot and root As concentrations in rice (Table 1). The shoot and root As concentrations of rice are shown in (Fig. 4a), and there was no significant difference between the shoot and root As concentrations of the CK and E treatments; AM treatment reduced shoot and root As concentrations, but did not reach significant levels ($P > 0.05$); EAM treatment reduced both shoot and root As concentrations by 38 % and 17 %, respectively. As accumulation in the shoot and root of rice showed a different pattern (Fig. 4b), which was related to the biomass, and the As accumulation in the shoot treatments was AM > E > EAM > CK. The As accumulation in

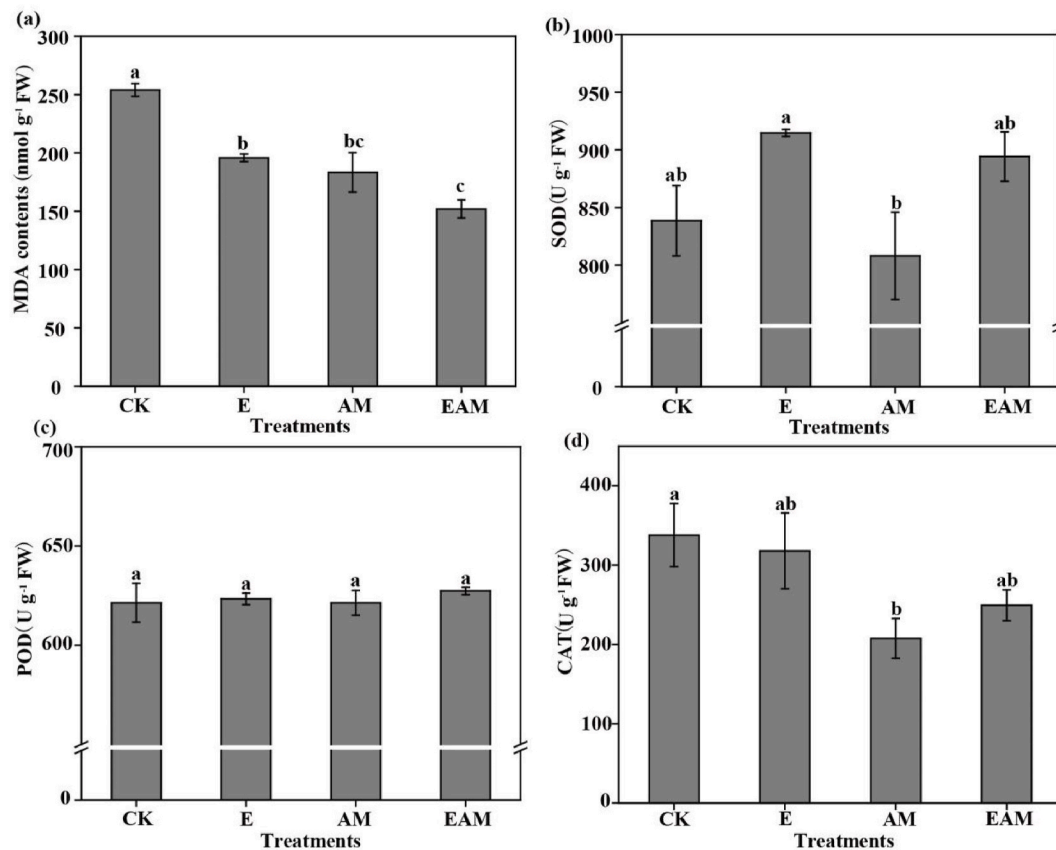


Fig. 2. Antioxidant indices of rice (a) MDA content, (b) SOD, (c) POD, and (d) CAT. Data are presented in means \pm standard error. Different lowercase letters show statistically significant differences among treatments ($P < 0.05$).

Table 2
Effects of earthworms and AMF on soil properties.

Treatments	pH	SOM (g kg ⁻¹)	AN (mg kg ⁻¹)	AP (mg kg ⁻¹)
CK	6.19 \pm 0.18a	11.42 \pm 0.08b	99.67 \pm 3.29c	42.87 \pm 0.90b
E	5.86 \pm 0.05b	9.58 \pm 0.14c	210.83 \pm 14.75a	42.23 \pm 0.81b
AM	6.45 \pm 0.05a	12.54 \pm 0.29a	114.02 \pm 6.60c	51.87 \pm 1.39a
EAM	6.34 \pm 0.03a	12.70 \pm 0.14a	175.30 \pm 5.88b	53.73 \pm 3.45a

Data are presented in means \pm standard error. Different lowercase letters show statistically significant differences among treatments ($P < 0.05$).

the root was $E > AM > EAM > CK$.

Further determination of subcellular distribution of As in the shoot and root, revealed that As was mainly concentrated in the cell wall (Fig. 4c). In leaves, compared to CK, the proportion of cell wall As concentration was increased by 3 %, 15 %, and 36 % in E, AM, and EAM treatments, respectively, with the EAM treatment reaching a significant level ($P < 0.05$); The proportion of soluble fraction As concentration was increased by 40 % and 26 % for AM and EAM treatments, respectively; For organelles, the proportion of As concentration was significantly decreased ($P < 0.05$) for both AM and EAM treatments, by 40 % and 48 %, respectively. A similar pattern was shown in the root, the EAM treatment significantly increased the percentage of cell wall As concentration compared to CK ($P < 0.05$); AM and EAM significantly decreased the proportion of organelle As concentration ($P < 0.05$), while there was no significant difference in the proportion of soluble fraction As concentration among treatments ($P > 0.05$).

3.5. PCA analysis and correlation analysis

In order to better determine the contribution of biological treatments to the growth and physiology of rice, PCA analysis was performed on all

growth and physiological indicators (Fig. 5a). PC1 and PC2 explained 35.2 % and 20.7 % of the variance, respectively, and separated by different treatments in different quadrants, indicating that earthworms and AMF significantly affected the growth of rice. E treatment was positively correlated with shoot N, root N, SOD, and chlorophyll, While AM treatment was positively correlated with shoot biomass, shoot P concentration, root P, CAT, and P/As. EAM treatment was positively correlated with the vast majority of indicators and negatively correlated with MDA and CAT. In summary, the EAM treatment contributed most to the growth of rice.

To clarify the relationship between As uptake in rice and the rhizosphere environment, the correlation of rice As accumulation and transport parameters with various indicators of the rhizosphere environment of rice was further analyzed (Fig. 5b). The shoot As concentration was positively correlated with As-F1 and As-F2, and negatively correlated with As-F3, pH, SOM, Catalase, Urease, Phosphatase, AK, AN, GRSP, suggesting that the bioavailable As in the soil is an important factor influencing As accumulation in rice.

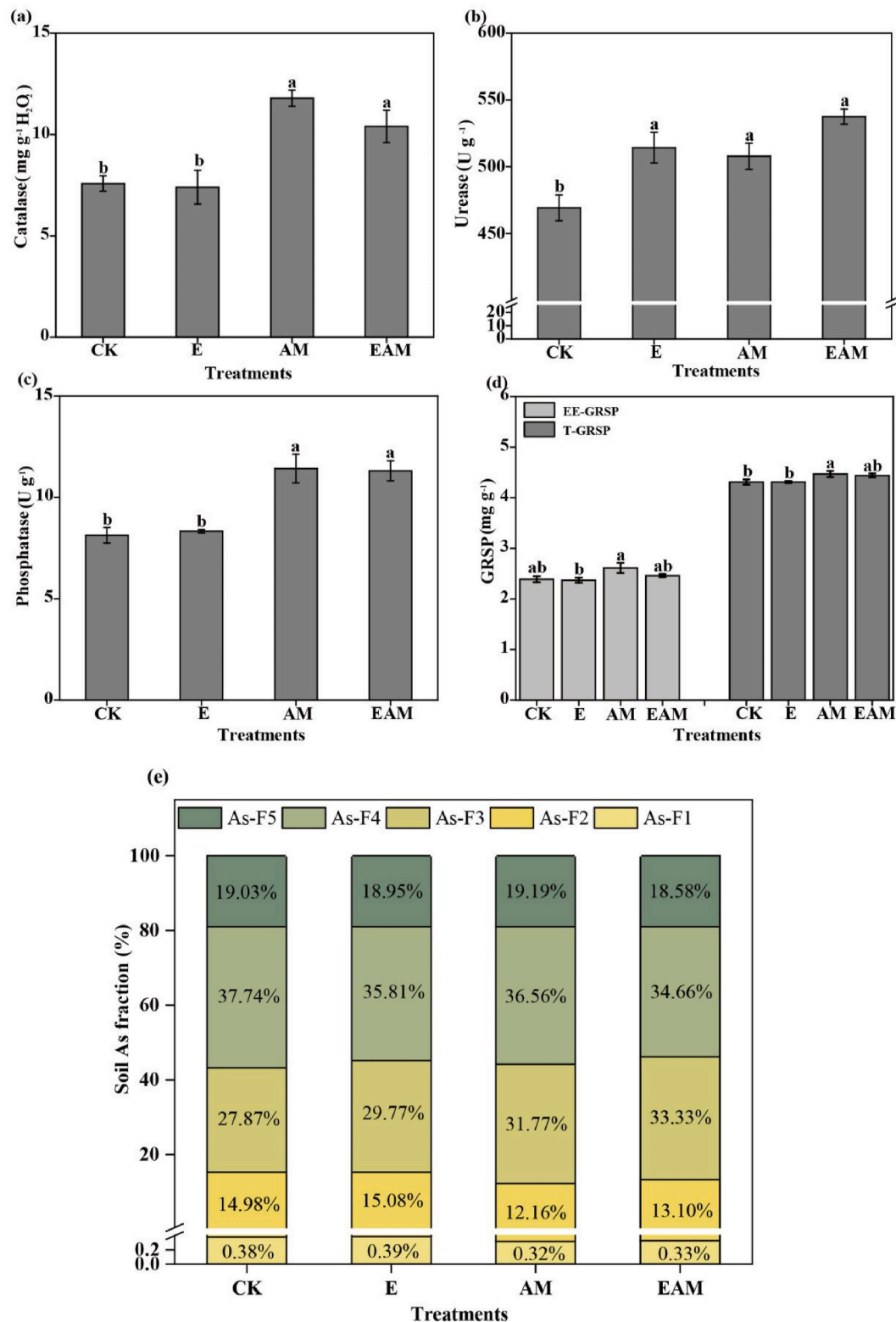


Fig. 3. Rhizosphere soil (a) catalase, (b) soil urease, (c) acid phosphatase, (d) GRSP, and (e) As fraction. Data are presented in means \pm standard error. Different lowercase letters show statistically significant differences among treatments ($P < 0.05$).

4. Discussions

4.1. Synergistic detoxification mechanisms

Plant tolerance is attributed to physiological functions in detoxification and transporter response to As. Mycorrhizal colonization rate,

plant biomass, nitrogen and phosphorus concentrations, chlorophyll content, and P/As are important plant growth indicators. The MDA content and antioxidant enzymes are important indicators of As tolerance in plants. In this study mycorrhizal colonization of rice was not higher (18–22 %) (Table S1), and in a non-flooded condition, the rate of AMF association was higher in rice and it showed 40 % colonization

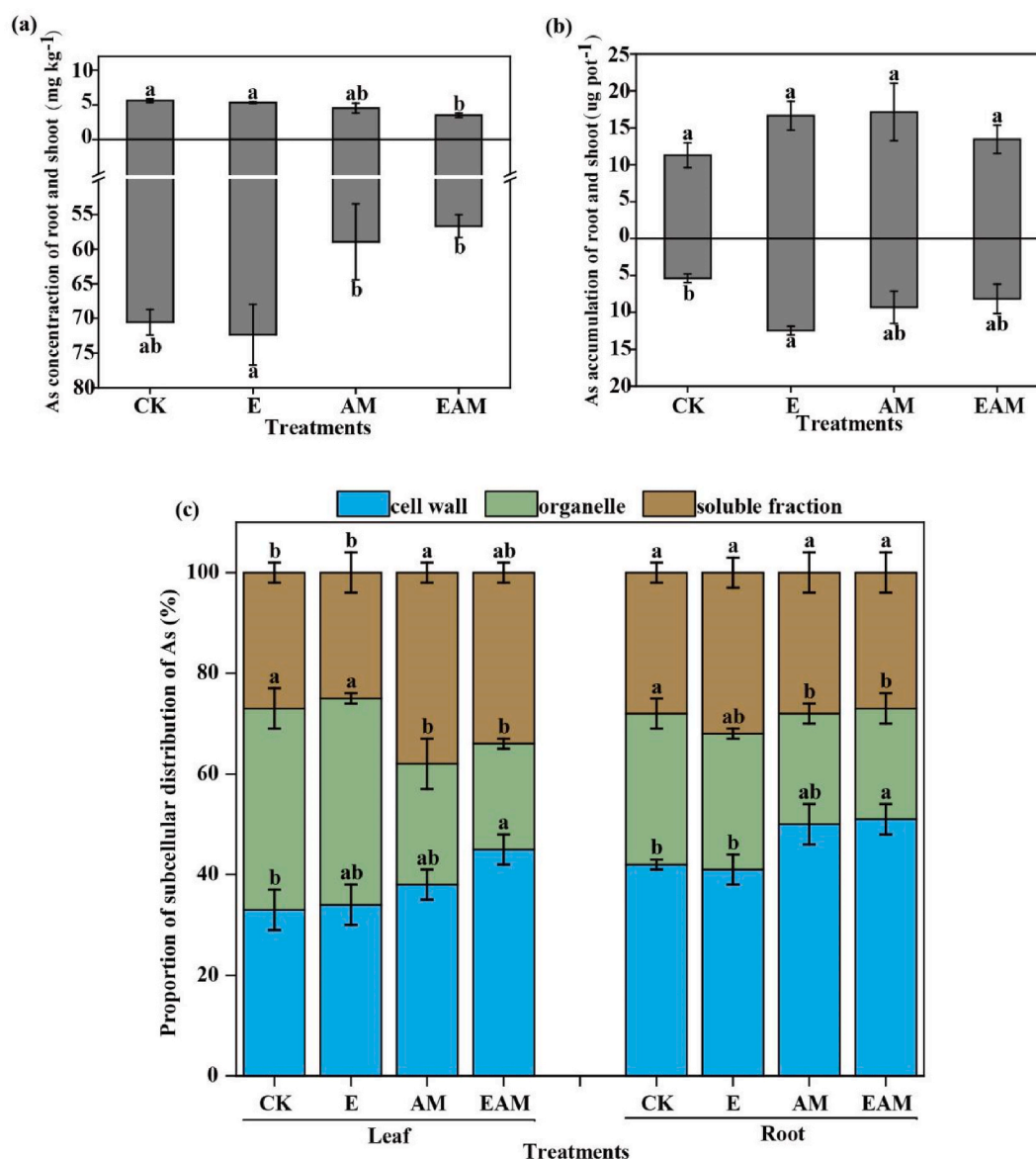


Fig. 4. As uptake in rice (a) As concentration, (b) As accumulation, and (c) proportion of subcellular distribution of As in shoot and root. Data are presented in means \pm standard error. Different lowercase letters show statistically significant differences among treatments ($P < 0.05$).

(Maiti et al., 2012). It is therefore hypothesized that this may be related to the relatively high levels of soil phosphorus (P) in this study, as mycorrhizae are more likely to form a symbiotic relationship with plants with low phosphorus levels (Kazantseva et al., 2009). Also, this may be related to high As concentrations in the soil (Li et al., 2016). Accumulation of toxic elements in the soil affects spore numbers and germination (Adeyemi et al., 2021), while root secretions reduce the transfer of carbonaceous compounds leading to a reduction in AMF colonization and inactivation in their root systems (Spagnoletti et al., 2017).

An indicator closely related to biomass is the growth dilution effect, which is an important mechanism for the detoxification of heavy metals in plants (Liu et al., 2005). In this study, AMF promoted rice growth more than earthworms, and the combined inoculation had the highest biomass inoculation effect, which is similar to the results of the previous study, and the reason for the improvement of plant growth by AMF and earthworms may be related to the improvement of the soil and the enhancement of the fast-acting nutrients (Kaur et al., 2017; Li et al., 2012). AMF and earthworms can stimulate plant growth by increasing nutrient acquisition. The N and P concentrations in the root and shoot of EAM were higher than those of the CK treatment in this study (Fig. 1c

and d). It may be one of the physiological mechanisms for increasing plant tolerance to heavy metal stresses (Leung et al., 2007). Among them, earthworms mainly had a significant effect on plant N uptake, while AMF had a significant effect on plant P uptake. This is similar to the study of (Ma et al., 2006), where earthworms and AMF were simultaneously inoculated into heavy metal contaminated soil, and the combined inoculation increased the N and P uptake capacity of silver acacia to promote growth. The increase in plant N and P uptake may be related to expanded uptake by AMF extraradical mycelia and increased soil urease and acid phosphatase activities, and the activation of N in the soil by earthworms may have reduced potential competition for N by AMF (Li et al., 2012).

One mechanism of action of As-induced plant poisoning is to cause disorganization of chloroplast structure and function (Dong et al., 2019). Chlorophyll can be used to reflect the ability to absorb and assimilate substances. In this study, inoculation with earthworms significantly increased the chlorophyll content of the plants and reduced the level of As toxicity, which may be related to the mucus secreted by earthworms (Kaur et al., 2019). In addition, the plant P/As is an important indicator of plant As tolerance. P and As exhibit a competitive

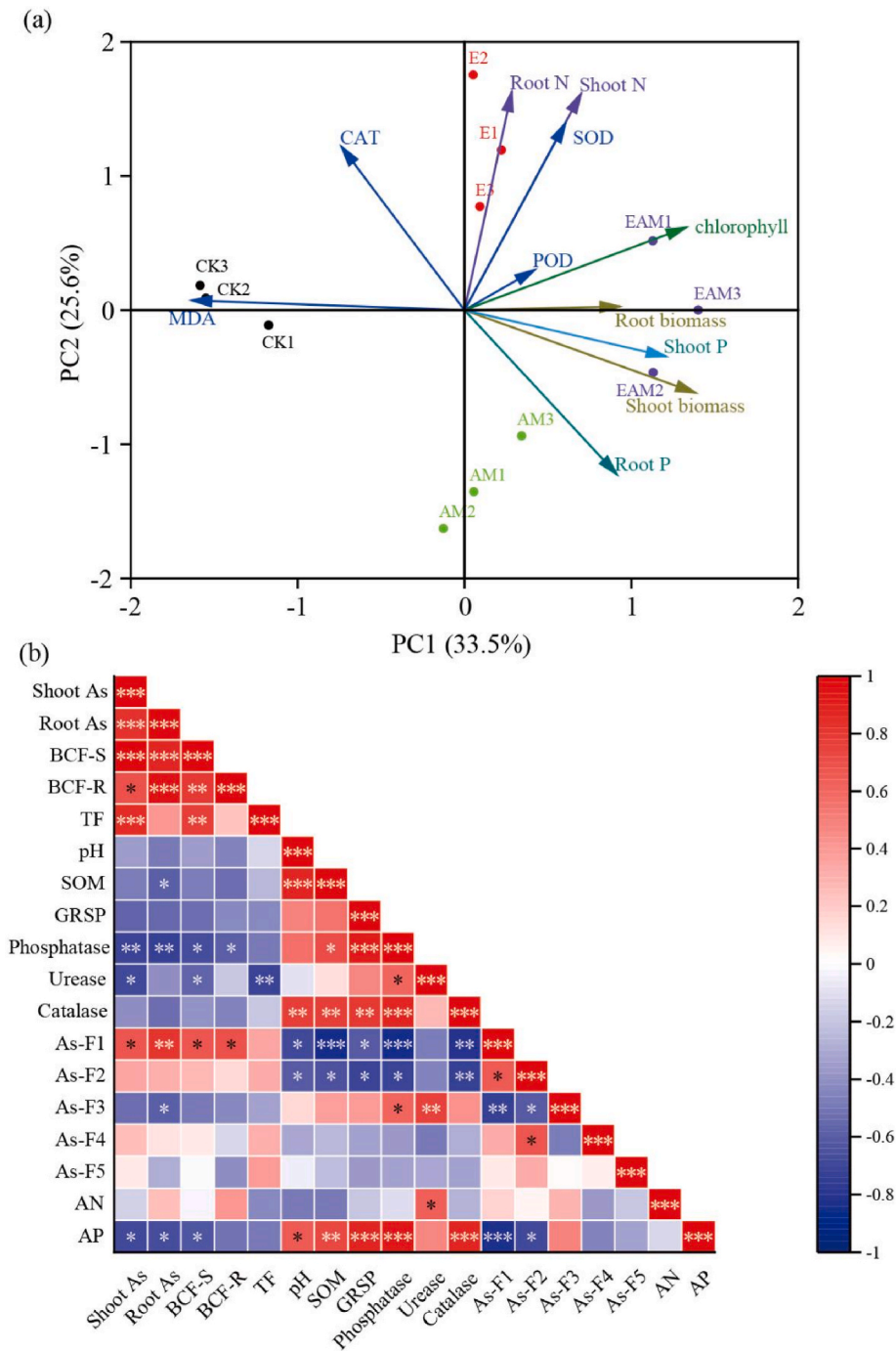


Fig. 5. (a) PCA analysis of growth physiological parameters, and (b) correlation heatmap of each indicator. *, ** and *** indicate significance at $P < 0.05$, $P < 0.01$ and $P < 0.001$, respectively.

relationship, they are very similar in the soil and share a common plant uptake pathway. In this study, the P/As increased after inoculation with earthworms and AMF, indicating that P uptake and translocation by plants was higher than As, which is in agreement with the results of previous studies (Chen et al., 2007). From a molecular mechanism perspective, previous studies have demonstrated that AMF inoculation can upregulate low-affinity Pi transporters in plant roots, thereby reducing As uptake while preferentially enhancing phosphorus (Pi) absorption (Gong et al., 2024). Although our study did not include specific gene expression analysis, the observed changes in P/As ratios align with this molecular mechanism, suggesting a similar regulatory pattern may be at play. It can be confirmed that earthworms and AMF enhance the As

tolerance of upland rice through nutrient-mediated growth dilution and competitive uptake.

Excess As causes oxidative damage and lipid peroxidation in plants. MDA is used as a measure of the extent of lipid peroxidation damage and is commonly used as a physiological indicator of plant exposure to stressful environments (Shah et al., 2020). In this study, all inoculation treatments reduced the MDA content of rice, which corresponded to the reduction of As concentration in rice, indicating that both earthworms and AMF can significantly alleviate the adverse effects of As stress on rice and maintain the stability of physiological activity. SOD, POD, and CAT are important antioxidant enzymes in plants, they have the role of scavenging and reducing reactive oxygen radicals, which can protect

cells from damage and is an important mechanism of plant detoxification (Li et al., 2020; Wang et al., 2021). The treatment inoculated with earthworms increased SOD, whereas AMF had no significant effect. There were no significant differences in POD among treatments, indicating that bioinoculation did not affect POD. Inoculation with AMF decreased CAT, and two-way ANOVA showed that AMF significantly affected CAT activity. In conclusion, earthworm mucus and AMF secretions likely stabilized reactive oxygen species (ROS) by enhancing SOD activity and reducing MDA content. This aligns with the antioxidant synergy hypothesis, where symbiotic organisms collectively buffer oxidative stress through complementary enzymatic pathways (Wang et al., 2021).

Clarifying the pattern of As uptake and transport in rice is important to As detoxification. In this study, AMF significantly affected shoot and root As concentrations in rice, and treatments inoculated with AMF (with or without added earthworms) showed a significant reduction in As concentrations, which may be related to the growth dilution effect and direct As immobilization by extraradical mycelia of AMF (Wang et al., 2005). It is worth noting that the role of earthworms in this may be to promote plant growth and enhance plant resistance while having no effect on plant As uptake. The analysis of As accumulation of rice showed that the treatments inoculated with AMF had lower As concentrations, but their large biomass caused their As accumulation to be larger than that of the CK treatment as well, which may be conducive to the enrichment of soil As in the root to achieve the effect of phytostabilization. To better analyze the effects of biological factors on As uptake and transport in rice, the Bioconcentration Factor (BCF) and Transport Factor (TF) were calculated (Table 3). Earthworms did not significantly affect BCF, while AMF significantly affected BCF-S and BCF-R, and there was a tendency for BCF to decrease in EAM treatments compared to CK. In addition, EAM treatment had the lowest TF ($P < 0.05$), and AMF inhibited the translocation of As from the root to the shoot. Studies have reported that AMF can promote the sequestration of As in root vacuoles by upregulating vacuolar chelation-related genes (e.g., OsABCC1), while simultaneously inhibiting the translocation of As from roots to shoots (e.g., through the action of OsPT1-13) (Christophersen et al., 2012). These findings are consistent with the observed changes in the As TF values in this study. AMF inoculation limits the transfer of heavy metals to the shoot through two mechanisms: the sequestration of metallic elements by AMF itself and the dilution effect on heavy metals by promoting plant growth (Chen et al., 2018; Liu et al., 2015). To further investigate the mechanism of As detoxification in rice, the subcellular distribution of As in leaves and roots was analyzed. In this study, AMF was a significant factor affecting the subcellularity of As. AM and EAM Treatments increase As proportion of cell wall and soluble fraction and decrease As proportion of organelle in leaf; In the root, AM and EAM treatments increased the As proportion of cell wall, decreased the As proportion of organelle, and had no effect on the As proportion of soluble fraction. Cell organelles are the most sensitive and vulnerable parts of the plant, and cell wall sequestration and vesicular compartmentalization prevent As from entering the site, which is essential for As detoxification (Kuang et al., 2022; Pandey and Khare, 2024; Wang et al., 2018).

Overall, AMF plays a dominant role in enhancing arsenic resistance in upland rice, whereas earthworms contribute by amplifying the

growth-promoting effects of AMF and improving the rhizosphere environment through nutrient activation, thereby further strengthening As tolerance.

4.2. Soil health and As stabilization

Plant growth is closely related to soil biology and soil properties, and the maintenance of soil species diversity and nutrient levels is essential for plant growth and their resistance to heavy metals. Soil enzyme activity and soil mineral nutrients are key factors indicative of soil health, and research on AMF and earthworms for improving soil properties has been widely reported (Chen et al., 2023; Yang et al., 2024). However, little research has been done on the combination of AMF and earthworms under high As stress, and their interactive effects have great potential to improve soil health. Soil pH has a great influence on the morphology, effectiveness, and transformation of heavy metals in soil. In this study, the earthworm treatment significantly reduced soil pH, which may be related to the acetic acid and oxalic acid secreted by earthworms. In contrast, inoculation with AMF (with or without earthworms) increased soil pH, which may increase the available As concentration. However, the available As concentration decreased in this study, suggesting that the mechanism by which earthworms and AMF affect soil As fraction is more complex, and that pH is just one of the factors. SOM not only affects soil function by improving soil properties and structure but is also one of the key factors influencing the bioavailability of heavy metals (Shi et al., 2024). Two-factor ANOVA showed that earthworm and AMF interaction had a significant effect on SOM, and earthworm activity resulted in a tendency for SOM to decrease, which may be related to the mucus secreted by earthworms stimulating SOM mineralization (Zhao et al., 2017). And AMF can increase SOM levels by releasing secretions rich in carbon compounds (Li et al., 2019).

Soil N and P are essential components for plant growth, and a previous study concluded that earthworms and AMF provide nutrients and establish distinct ecological niches for plant growth (Li et al., 2019). Earthworms contributed more to AN in this study, while AMF contributed more to AP. Earthworms and AMF provide different nutrients to the soil and plants, respectively, with complementary effects. Increased soil AN by earthworm activity may be related to nitrogen metabolites produced by protein breakdown in earthworm feces, mucus, and ureases (Padmavathiamma et al., 2008). The increase in AP may be related to organic acids, and acid phosphatase (Mahohi and Raiesi, 2021). Soil enzymes can be used to indicate soil health and microbial activity (Di et al., 2019; Niu et al., 2021). Catalase activity can reflect the degree of heavy metal pollution to a certain extent (Zhang and Ji, 2019). In this study, AMF was more effective than earthworms in promoting soil catalase activity, suggesting that AMF is beneficial in alleviating the toxic effects of As. Urease activity increased with the addition of earthworms, which is similar to the findings of (Aghababaei et al., 2014a,b). Earthworms enhance soil enzyme activity by accumulating heavy metals in tissues, inducing microbial growth ultimately (Boughattas et al., 2019). Increased acid phosphatase activity by AMF may be related to the secretion of extraradical mycelia and its amelioration of rhizosphere microbial activity (Berthelot et al., 2018; Sato et al., 2019). In addition, AMF significantly affected all three soil enzyme activities in this study (Table 1), whereas earthworms mainly affected soil urease, suggesting that AMF was the main biological factor affecting soil enzyme activities.

AMF can secrete GRSP which is usually divided into two fractions: easily extractable GRSP (EE-GRSP) and total GRSP (T-GRSP) (Jia et al., 2016). It has been shown in many studies that they play an important role in binding heavy metals (Ji et al., 2019; Malekzadeh et al., 2016). The results of this study showed that AMF treatment significantly increased T-GRSP content, and the increase in GRSP content was correlated with AMF extraradical mycelia (Zhang et al., 2020). found that GRSP secreted by AMF enhanced P uptake and reduced As toxicity

Table 3

Effects of earthworm and AMF on Bioconcentration Factor (BCF) and Translocation Factor (TF) in rice.

Treatments	BCF-shoot	BCF-root	TF
CK	0.022 ± 0.001a	0.276 ± 0.010a	0.079 ± 0.002a
E	0.022 ± 0.001a	0.294 ± 0.022a	0.074 ± 0.005a
AM	0.018 ± 0.003 ab	0.231 ± 0.028a	0.076 ± 0.006a
EAM	0.015 ± 0.001b	0.246 ± 0.008a	0.060 ± 0.004b

Data are presented in means ± standard error. Different lowercase letters show statistically significant differences among treatments ($P < 0.05$).

in acacia seedlings. Correlation analysis showed that GRSP was negatively correlated with As-F1, As-F2 and As concentration in rice (Fig. 5b), which indicated that GRSP stabilized soil As in this system to some extent.

Heavy metal forms and their distribution patterns in soils determine their bioavailability and their homing behavior and toxicity in soil-plant systems (Jusselme et al., 2013; Setia et al., 2021). Two-factor ANOVA showed AMF significant effects on As-F1, As-F2, and As-F3. AMF promotes the conversion of As-F1 and As-F2 to As-F3, so that the conversion of As to a more insoluble form is conducive to the effect of phytostabilization, thereby reducing the accumulation of As in the shoot of the plant. It has been shown that AMF can alter the activities of soil enzymes and microorganisms, as well as the physical and chemical environment of soil, which can affect the transport and morphological distribution of heavy metals in soil (Aghababaei et al., 2014a,b). Similarly, earthworms can alter the effectiveness and component patterns of heavy metals in soil, and the mechanism may be related to the regulation of soil pH, and organic matter, stimulation of soil microbial populations, and the transformation and storage of heavy metals in earthworms. However, no significant effect of earthworm inoculation was observed in this experiment, which may be related to the soil and plant type.

It can be confirmed that the observed increase in soil enzyme activities and SOM aligns with the Soil Health Index (SHI) framework, which emphasizes microbial functionality as a key indicator of soil resilience (Guo et al., 2019). These enzymes mediate nutrient mineralization and detoxification, reflecting the principle that healthy soils maintain robust biogeochemical cycles under stress (Antoniadis et al., 2019). Furthermore, the reduction in bioavailable As fractions supports phytostabilization strategies, reducing entry of As into the food chain - a critical goal of the One Health initiative (Abedi and Mojiri, 2020).

5. Conclusions

This study reveals that earthworm-AMF co-inoculation synergistically enhances rice tolerance to As through integrated plant-soil mechanisms. First, co-inoculation increased rice biomass and nutrient uptake (N/P), concurrently reducing As uptake in shoot and promoting vacuolar sequestration of As in roots. This growth dilution effect alleviates the toxicity of arsenic to rice. Second, the treatment alleviated oxidative stress by boosting antioxidant enzymes. Critically, soil health was improved through pH modulation, SOM enrichment, and reduced As bioavailability. These findings establish a novel tripartite interaction system (earthworm-AMF-rice) for As mitigation, offering a low-cost bioremediation strategy for contaminated soils. While this study demonstrates correlational linkages, future work should dissect causal mechanisms via transcriptomic analysis of As transporters and functional profiling of the rhizosphere microbiome.

CRedit authorship contribution statement

Zipeng Chen: Writing – original draft, Formal analysis, Data curation, Conceptualization. **Wanlin Li:** Writing – review & editing. **Rakhwe Kama:** Writing – review & editing. **Farhan Nabi:** Investigation. **Zhansheng Kou:** Investigation. **Rongliang Qiu:** Conceptualization. **Xu Yang:** Writing – review & editing, Formal analysis, Data curation. **Huashou Li:** Writing – review & editing, Investigation, Funding acquisition.

Declaration of competing interest

The authors declare that they have no conflict of interest.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.jenvman.2025.125213>.

Data availability

The authors do not have permission to share data.

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The potential of earthworms and arbuscular mycorrhizal fungi to enhance phytoremediation in heavy metal-contaminated soils: a review

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Abstract

Earthworms and arbuscular mycorrhizal fungi (AMF) are two different organisms playing crucial role in soil mechanisms. The integration of earthworms and AMF in phytoremediation strategies leverages their combined ability to improve soil structure, nutrient availability, and microbial activity while modulating metal bioavailability. These entities promote soil-plant interactions and enhance the phytoremediation process of heavy metals-contaminated soil. This review explores the mechanisms by which earthworms and AMF function individually and in combination in the phytoremediation of heavy metal-contaminated soil. The main objectives of this were determine earthworms heavy metals tolerance, absorption and transformation, as well as the synergistic effect between earthworms and plants. Further, the effects of AMF on heavy metals phytoremedoation process was also analyzed as well as the potential interactions between earthworms and AMF on heavy metals removal. This partnership can optimize plant health and remediation efficiency, making it a promising approach for restoring heavy metal-contaminated soils. Thus an integrated empirical study was conducted to summarize the effects earthworms and AMF interactions on heavy metals phytoremediation and to highlight the impact of their individual and combined actions on the phytoremediation paramters. Avenue for further studies towards improved phytoremediation process we discussed. This review emphasize that earthworms and AMF can be employed as biological method to enhance the phytoextraction by hyperaccumulator plants on severely heavy metal-contaminated soil. Alternatively, in moderately and lowly contaminated farmland, the transfer of heavy metals to the above-ground parts of crops can be reduced to promote safe production.

Keywords Earthworms · Arbuscular mycorrhizal fungi · Phytoremediation · Heavy metal pollution · Synergistic effect

Introduction

The rapid industrialization and urbanization in the last few decades have caused major environmental problems including soil pollution. For instance, it has been documented that industries, chemical fertilizers and pesticides, as well as waste water are the main sources of heavy metals in the environment. In addition, the release of heavy metals affects negatively soil texture and functions (Han et al. 2002; Nogawa et al. 2017). Moreover, previous studies showed that heavy metals accumulation in soil poses significant threat to human health and other organisms through the biomagnification in the food chain (Antoniadis et al. 2019; Khan et al., 2021). It is estimated that nearly 20% of farmland soil in China has been contaminated by heavy metals. Among these, cadmium pollution is the most severe, with an

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exceedance rate of 7.0% at sampling sites, while the exceedance rate for arsenic is 2.7% (Sun et al. 2019). These statistical data indicate that heavy metals concentration in China's agricultural soils have significantly increased, serving as a warning for sustainable agriculture.

Heavy metals remediation in cultivable land is a crucial and urgent challenge that needs to be won. At present, there are three methods for heavy metal remediation: physical, chemical, and biological. Traditional physical and chemical methods are characterised by high costs, high energy consumption, and environmental impact unfriendly. In contrast, bioremediation has the advantages of low cost, environmentally friendly, simple operation and no occurrence of pollution migration. Phytoremediation is a technique that utilizes plants to remove toxic and harmful substances or reduce their harmful effects. According to the purification methods, it can be divided into phytostabilization, phytoextraction and phytoexclusion (Laghlimi et al. 2015). Soil pollution is very complex and requires multiple actions to achieve the remediation process. Therefore, several scientists suggest the combined remediation which refers to a remediation method that uses different remediation techniques together due to the complexity of soil pollution. Therefore, the plant-animal-microbe combined remediation technique is adopted to deal with complex heavy metal pollution situations (Udovic and Lestan 2007).

AMF can increase the colonization capacity of plants, facilitate plant growth, and promote the accumulation of heavy metals by plants, which highlights the effectiveness of plant-mycorrhizal fungi combined remediation as a meaningful and reliable strategy (Liu et al. 2020a, b). Leveraging the symbiotic relationship between AMF and plant roots, the function of AMF is incorporated into various remediation processes such as phytoextraction and phytostabilisation to enhance remediation efficiency and bolster the overall remediation effect (Artursson et al. 2006). Plants supply carbohydrates to AMF, while AMF reciprocally enhance root nutrient and water acquisition. This symbiosis critically supports ecosystem stability and biodiversity through optimized resource exchange (Gao et al. 2023). Many existing studies have shown that AMF and their extraradical mycelium can regulate the adsorption of heavy metals (Bhargava et al. 2016; Riaz et al. 2021; Trouve et al. 2014; Zhang et al. 2018). However, multiple organisms often coexist within the soil ecosystem. For instance, earthworms, which are soil-dwelling animals, do not have a defined relationship of upper and lower trophic levels, yet they may influence the formation of mycorrhizal symbionts through bioturbation and physiological processes (Paudel et al. 2016). Existing studies present varied findings regarding the impact of earthworms on mycorrhizal colonization, including promotion (Trouve et al. 2014), inhibition

(Lawrence et al. 2003), or no effect impact (Wurst et al. 2004). There is still no definite conclusion on how earthworms affect AMF. Moreover, earthworms, often referred to as “soil ecological engineers”, not only mix and transfer soil components but also enhance soil fertility by absorbing partially decomposed substances from the soil surface and transporting them to the subterranean layer. Research has demonstrated that earthworms can sequester heavy metals within their tissues, indicating their potential use as biological indicators and in the bioremediation of contaminated soil (Hockner et al. 2015; Sizmur and Hodson 2009). Currently, there are very few studies on the synergistic mechanism of AMF and earthworms under heavy metal stress. This paper analyses the individual impacts of earthworms and AMF on phytoremediation, while dissecting the potential of the combined effect of earthworms and AMF on phytoremediation in heavy metal-contaminated soil, to provide new insights into phytoremediation.

The role of earthworms on the phytoremediation in heavy metal-contaminated soil

Earthworms are one of the common organisms in the soil ecosystem playing a vital role in improving soil quality (Cheng et al. 2021). Meanwhile, earthworms demonstrated a certain tolerance to heavy metals and can survive in soil with a degree of pollution (Chai et al. 2020). Their behaviours often impact on the migration and transformation of heavy metals in the soil (Jusselme et al. 2013). Consequently, many studies already regarded earthworms as a bioremediation method for threatening heavy metals in the soil (Nannoni et al. 2011; Wu et al. 2020; Zeb et al. 2020). The relationship between earthworms and the remediation of heavy metals in soil is shown in Fig. 1.

The tolerance of earthworms to heavy metals in soil

Earthworms counteract heavy metals by detoxifying, regulating and eliminating them. In addition, earthworms can survive in highly contaminated soil with heavy metals and absorb a certain amount of heavy metals mainly by relying on antioxidant enzymes, bioactive molecules and coelomic fluid to relieve the oxidative stress caused by heavy metals (Chen et al. 2017). Although oxidation is an important chemical reaction in organisms, balancing the oxidation reaction is an important mechanism to prevent free radicals from damaging cell functions. In this way, the balance of the oxidation reaction is achieved through the synthesis of

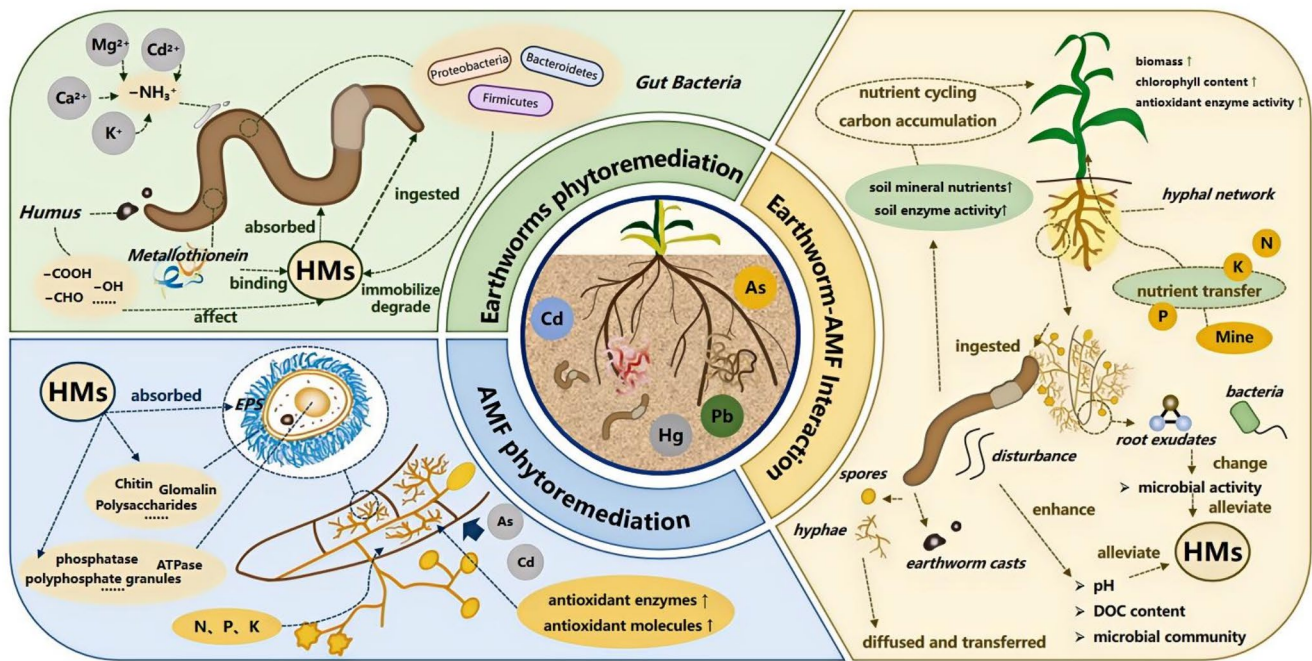


Fig. 1 The roles of earthworms and AMF in the phytoremediation of heavy metals in soil. Earthworms phytoremediation: Earthworms promote soil nutrient cycling and enhance root system development through their bioturbation activities, mucous secretions, cast deposition, and gut microbial interactions, while simultaneously altering the chemical speciation and spatial distribution of heavy metals in soil ecosystems. AMF phytoremediation: AMF not only mediate heavy metal adsorption and chelation through their mycelial architectures and

extraradical hyphae networks, but also modulate metal speciation via secretory exudates, while concurrently enhancing plant antioxidative defense. Earthworm-AMF phytoremediation: Earthworms enhance AMF spore dispersal and root colonization while stimulating soil enzymes to strengthen mycorrhizal networks and nutrient exchange. Their bioturbation interacts with AMF metabolites to reshape microbial communities and reduce heavy metal bioavailability through microbial redox processes and organic complexation.

antioxidants such as catalase, superoxide dismutase (Li et al. 2017a, b).

Many metals can bind to metallothionein (MT), a low-molecular-weight protein that regulates the dynamics of bioavailability and detoxification of essential and non-essential metals in the earthworm gut (Bhattacharya and Kim 2016; Maity et al. 2011). MT can provide cysteine thiolate ligands and serves as a metal buffer in cells. It plays a dual role by both storing biologically essential metals and sequestering toxic metals. The regulation of MT is evolutionarily conserved across the intestinal epithelium, coelomocytes, kidneys, and cecum of various earthworm species. This regulation is involved in the binding of metal transcription factor 1 (MTF-1) to the metal response element (MRE) located in the MT promoter (Hoeckner et al. 2015). Some studies have put forward the hypothesis that earthworms living on the soil surface (epigeic species) can produce a large amount of humus. These humus can stimulate the exchange process of heavy metal ions in the soil, making them bind to MT in earthworms, thus reducing the metal content in the soil (Yuvaraj et al. 2021).

Earthworm's absorption and transformation of heavy metals in soil

Earthworms are in close contact with soil pollutants and can tolerate heavy metals. They typically absorb heavy metals through two main processes. There are two main ways that pollutants in the soil can affect earthworms. First, pollutants can be easily absorbed through their skin (epidermis). Second, earthworms can consume a significant amount of soil particles, which allows them to take in the pollutants that are attached to those particles (Oste et al. 2001; Saxe et al. 2001).

The effect of earthworms on heavy metal availability in soil can be categorized into direct and indirect influences. Earthworms impact the bioavailability of heavy metals through the secretion of mucus and castings. For instance, mucus secreted by earthworms contains amino acids with positive (amino groups) and negative (carboxyl groups) sites. The charged parts of the amino acids in the mucus can bind to soil particles and metal cations, forming metal complexes to prevent their movement (Zhang et al. 2020). Earthworm casts contain a large amount of humus which plays a crucial role in the soil system by improving soil fertility and regulating heavy metals availability. The functional

groups of humic acid can affect the forms of heavy metals and effectively control heavy metal pollution in farmland soil (Botero et al. 2010; Zhang et al. 2019). Sizmur and Richardson (2020) pointed out that the introduction of earthworms might increase the mobility of heavy metals and improve the availability of heavy metals. Vanhoudt et al. (2024) found that the presence of earthworms significantly increased the concentrations of most heavy metals in the soil pore water, which might be caused by the decrease in pH and the enhancement of humification. Besides, earthworm casts contain bacteria, actinomycetes and fungi. These microorganisms degrade organic matter in the soil through enzymatic reactions, releasing heavy metals bound to that organic matter into the soil solution, which increases the bioavailability of these metals (Wen et al. 2004).

The skin of earthworms functions as a biological filter for heavy metals in the soil, while the intestine serves as a biological converter (Gudeta et al. 2023). Earthworm gut bacteria not only accumulate heavy metals but also play a crucial role in the bioremediation of these metals (Krautkramer et al. 2021; Wang et al. 2019). The bacterial community in the earthworm gut is significantly different from that in the surrounding soil. For instance, relative abundances of *Bacteroidetes*, *Proteobacteria* and *Firmicutes* in the earthworm gut are relatively high (Liu et al. 2020a, b). Some studies have found that earthworm gut bacteria can promote the transformation of arsenic in the soil by enhancing the arsenic reduction and methylation functions of the soil microbial community. The synergistic effect of earthworm gut and soil microorganisms increases the abundance of arsenic metabolism genes and accelerates the removal of arsenic from the soil (Wu et al. 2024). All in all, the gut bacteria of earthworms show great capabilities in promoting plant growth and removing toxic trace elements in the environment (Banerjee et al. 2019).

The activities of earthworms will have an important impact on the physical and chemical properties of the soil and the rhizosphere microbial community, and then indirectly affect the bioavailability of heavy metals (Ma et al. 2002; Sizmur and Hodson 2009; Yu et al. 2005). Earthworm mucus can increase soil pH due to its relatively high content of nitrogenous substances and the secretion mechanism of calciferous glands, thus reducing the availability of heavy metals (Bityutskii et al. 2012). Wang et al. (2023) found that earthworms can increase the abundance of ammonia-oxidizing bacteria (AOB) in the soil and promote soil nitrification, which leads to a decrease in soil pH and an increase in bioavailability. Wen et al. (2004) put earthworms into the soil with heavy metal pollution and the soil without heavy metal pollution respectively, and found that there was a significant positive correlation between the content of heavy metals and dissolved organic carbon (DOC) in the water

of the heavy metal-treated soil. Many similar studies have also shown that the presence of earthworms increases the DOC in the soil. DOC can complex with heavy metals in the soil or bring heavy metals into the soil solution through exchange reactions, thereby enhancing the bioavailability of heavy metals (Evangelou et al. 2004; Udovic and Lestan 2007).

The synergistic effect of earthworms and plants on heavy metals-contaminated soil remediation

Besides removing heavy metals in the soil through direct absorption, earthworms also participate in and influence the absorption of heavy metals by plants. The efficiency of single phytoremediation is low due to slow plant growth, low biomass, poor tolerance to combined pollution and low availability of heavy metals in the soil (Ali et al. 2013). The increase in plant biomass promoted by earthworms is an important reason for enhancing the efficiency of phytoremediation. The mechanisms include the following four aspects: (i) suppressing diseases and pests, (ii) stimulating soil microorganisms, (iii) producing plant growth-promoting molecules, (iv) increasing nutrient bioavailability, and (v) improving soil structure (Hullot et al. 2021). Substantial empirical evidence demonstrates that earthworm bioturbation and secretions significantly facilitates metal (loid) mobilization through enhanced leaching processes, accelerates their geochemical dispersion, and promotes trophic transfer pathways within ecosystems. This biologically mediated redistribution mechanism critically optimizes phytoextraction efficiency by increasing heavy metal bioavailability in the rhizosphere (Sizmur and Hodson 2009; Vanhoudt et al. 2024). *Lantana camara* is a hyperaccumulator of Pb. Inoculating earthworms can increase the biomass of *Lantana camara* by 1.5 to 2 times, increase the lead absorption amount by 2 to 3 times, and the removal rate of lead in the soil can be increased from 10 to 20% (Juselme et al. 2012). Hullot et al. (2021) found that plants and earthworms can affect soil properties, soil pore water and the migration of toxic elements. The interaction between earthworms and plants has changed the bioavailability of zinc by increasing the zinc concentration in earthworms and reducing the zinc concentration in plants.

The inoculation of earthworms is also beneficial for enhancing plants' resistance to heavy metals. Cadmium has a high affinity for S and N atoms in the side chains of amino acids, and amino acids can alleviate cadmium toxicity in plants by chelating cadmium ions to form complexes (Wei et al. 2003). In addition, earthworm mucus also improves the chlorophyll content, antioxidant enzyme activity and the

absorption and transport capacity of essential trace elements in plants, which may be related to its containing substances similar to auxin (IAA) (Wu et al. 2004; Zhang et al. 2009a, b). Similar studies have found that both earthworm mucus and amino acids significantly increase the concentration of cadmium stored in the soluble fraction of sub-cells as well as the concentrations of inorganic and soluble forms of cadmium in tomato seedlings (Zhang et al. 2009a, b).

The role of AMF on the phytoremediation of heavy metal-contaminated soil

AMF have both direct and indirect effects on phytoremediation. The reaction mechanism between AMF and heavy metals in soil is shown in Fig. 1.

The direct effect of AMF on the phytoremediation

AMF chelate and sequester heavy metals through their fungal structures. Meanwhile, the glomalin secreted by them can also immobilize heavy metals, which is the first physical barrier for AMF to directly influence the entry of heavy metals into plants (Riaz et al. 2021). The fungal structures of AMF include arbuscules, cytoplasm, vacuoles and cell walls. These structures can immobilize heavy metals, reduce their transfer to the above-ground parts and enhance plant resistance (Wu et al. 2015). Chitin, glomalin and polysaccharides in the cell walls of AMF can regulate the adsorption of heavy metal ions. Various functional groups such as imidazole carboxyl groups, amino groups and free hydroxyl groups can provide binding sites for heavy metals, thus forming negatively charged structures with them. The polyphosphate granules, vacuolar proton ATPase and vacuolar proton phosphatase in vacuoles contribute to the adsorption of heavy metals by vacuoles (Abdelhameed and Metwally 2019; Shi et al. 2019). Chen et al. (2018) have observed through synchrotron radiation micro X-ray fluorescence (SR- μ XRF) imaging that arbuscules and intercellular hyphae of AMF have high Cd accumulation, and Cd is mainly retained in the arbuscules of the fungal structures. However, some studies have observed that Cd, Cu and Zn in the extraradical hyphae and spores of fungi are mainly distributed in the cell walls and vacuoles of the fungi (Wu et al. 2018).

In addition, the surface of AMF hyphae can produce extracellular polymeric substances (EPS). EPS has functional groups such as carboxyl groups, amines, phosphoric acid and hydroxyl groups, and it can adsorb metals through chelation, surface precipitation and ion exchange (More et al. 2014). Glomalin-related soil protein (GRSP) is a metal-tolerant glycoprotein secreted by AMF. It can chelate heavy metals and enrich electron ligands to reduce the

bioavailability of heavy metals (Jia et al. 2018). Wu et al. (2014) found that the GRSP absorbed 4% of the Pb and Ni in the soil after 140 days. Similarly, studies have shown that 1 gram of GRSP can extract up to 4.3 milligrams of Cu, 0.008 milligrams of Cd and 1.12 milligrams of Pb from contaminated soil. The bioavailability and mobility weakened due to the complexation of GRSP with metals are beneficial to the phytostabilization process (Gonzalez-Chavez et al. 2004). Finally, the transporters existing in the hyphae of AMF play an important role in alleviating heavy metal toxicity and the process of heavy metal compartmentalization. For example, metallothionein in the AMF structure can chelate heavy metals through the sulfhydryl groups provided by cysteine, thereby reducing the bioavailability and transport capacity of heavy metals (Lenoir et al. 2016).

The indirect effects of AMF on the phytoremediation

The indirect effects of AMF on enhancing phytoremediation are mainly achieved by promoting plant nutrient acquisition, enhancing plant antioxidant capacity and regulating gene expression at the gene level (Wu et al. 2013, 2019; Zou et al. 2019).

AMF can improve soil conditions and then promote plant growth (dilution effect). AMF has an important impact on the biogeochemical cycle of phosphorus in the soil. AMF can cause a deficiency of phosphorus in the soil around the extraradical hyphae (Li et al. 2017a, b). The extraradical hyphae grow away from the phosphorus-deficient zone, increasing the absorption area of roots and enabling host plants to absorb phosphorus in a larger range (Whiteside et al. 2019). Some studies have stated that AMF extraradical hyphae can provide up to 80% of inorganic phosphorus (Wipf et al. 2019). After inoculating AMF into cotton, the expression of the phosphorus transporter gene in cotton was enhanced, and the phosphorus content in cotton was increased by 43.27% (Gao et al. 2020). Chang et al. (2018) found that through pot experiments under the stress of cadmium and lanthanum, AMF could increase the biomass of maize and the nutrients of N, P and K in plants (by 20.1–76.8%), while reducing the content of heavy metals in plants and alleviating toxicity.

Under heavy metal stress, plants generate excessive reactive oxygen species (ROS) that induce cellular and molecular alterations (e.g., downregulation or upregulation of gene expression), thereby triggering regulatory mechanisms to rapidly respond to these stress conditions (Luo et al. 2016). Excessive ROS can damage plant cells and then affect their normal physiological activities. However, inoculating AMF can promote the activities of antioxidant enzymes (such as superoxide dismutase, peroxidase, catalase, ascorbate peroxidase, etc.) and the synthesis of non-enzymatic antioxidants

(such as glutathione, ascorbic acid, phenols and carotenoids, etc.) to scavenge ROS and counteract the oxidative damage within plants (Li et al. 2020). Sharma et al. (2017) pointed out that compared with non-mycorrhizal plants, mycorrhizal plants have higher activities of antioxidant enzymes (superoxide dismutase, peroxidase and catalase) and antioxidant molecules (proline, carotenoids and α -tocopherol) under As stress, which significantly reduces the toxic effects of ROS. AMF can also regulate the expression of plant-related genes. After inoculating AMF, the expression of the PT4 gene can be up-regulated to help maintain the phosphorus status in roots. This symbiotic relationship can prevent the transfer of As to the aboveground parts of plants (Li et al. 2018). Similarly, after inoculating AMF, the expression of the HMA3 gene is up-regulated. The HMA3 gene can chelate Cd into the vacuoles of root cells and prevent it from migrating to the aboveground parts (Miyadate et al. 2011).

The capacity for heavy metal uptake and accumulation exhibits significant interspecific variation among plants, governed by host-specific physiological traits and inherent detoxification mechanisms, including differential expression of metal transporters and chelation processes. Chen et al. (2012) found that inoculating AMF onto rice in As-contaminated soil significantly reduced the As absorption of the rice. In contrast, another study reported that inoculating AMF onto ryegrass in Cd-contaminated soil increased the Cd content in the roots and shoots of ryegrass by 30% and 35%, respectively (Han et al. 2021). Therefore, the distribution of heavy metals in plants under the influence of AMF has two aspects. In the process of phytoremediation of heavy metals, applying AMF to hyperaccumulator plants and taking advantage of its characteristic of promoting the absorption of heavy metals by plants under certain conditions can improve the extraction efficiency of hyperaccumulator plants for heavy metals (Leung et al. 2010). For non-hyperaccumulator plants, inoculating AMF can both enhance the plants' absorption of heavy metals (Yin et al. 2021), and reduce the plants' absorption of heavy metals to play a protective role (Chen et al. 2012).

The role of the earthworm-AMF interaction on the phytoremediation of heavy metal-contaminated soil

Earthworms play an important role in terrestrial ecosystems by regulating the migration and transformation of heavy metals in the soil-plant system through activities such as feeding, metabolism, and excretion. AMF affects the absorption, accumulation, and detoxification of heavy metals by plants in heavy metal-contaminated soil through multiple pathways (Wang et al. 2022). Although earthworms and AMF directly interact with the rhizosphere of host plants, little

is still known about the interaction effects between these two important organisms, and it remains unclear how their interaction regulates the soil to affect plant functions. Fig. 1 describes the impact of the interaction between earthworms and AMF on the soil and plants. The following table summarizes the research on the impact of the earthworm-AMF interaction on phytoremediation in recent years (Table 1).

The impacts of earthworms on AMF

AMF and earthworms are at different trophic levels in the same ecosystem and there is no predator-prey relationship between them. Although earthworms do not mainly feed on fungal hyphae, they can selectively feed on fungal hyphae. On the one hand, they may damage the fungal hyphae and thus affect the availability of nutrients. On the other hand, they may also be beneficial to spore dispersion and root colonization (Meng et al. 2021). Some studies have claimed that the spores and hyphae carried in the earthworm gut can be diffused and transferred to new areas through the movement of earthworms and earthworm casts, which may promote mycorrhizal colonization of roots (Milleret et al. 2009). Pelosi et al. (2024) pointed out that earthworms can act as transporters of AMF in the soil, increasing the colonization rate and thus enhancing plant nutrition. In a latest study, adding earthworms to alfalfa under molybdenum stress led to a partial recovery in the formation of mycorrhizal symbiotic structures. Compared with the treatments without adding earthworms, the addition of earthworms increased the colonization rate of AMF by 9.4–33.6% (Yang et al. 2024). However, Dehghanian et al. (2018) found in a silt loam soil experiment that earthworms had no impact on the mycorrhizal colonization of maize. Aghababaei et al. (2014b) showed that the impact of earthworms on mycorrhizal colonization was related to the type of mycorrhiza. Earthworms were beneficial to the colonization of *Funneliformis mosseae* on the roots of maize, while earthworms had no impact on the colonization of *Rhizophagus intraradices*. Earthworms were beneficial to the colonization of *Funneliformis mosseae* on the roots of maize, while earthworms had no impact on the colonization of *Rhizophagus intraradices*. Some studies have found that without the addition of straw, earthworms significantly reduced the colonization rate of AMF, but this effect disappeared when straw was added (Ortiz-Ceballos et al. 2007). In addition, earthworms can promote the growth of fungal hyphae by increasing the biomass of host plants and activating soil carbon and nitrogen (Gormsen et al. 2004). According to the data statistics in Table 1, the results of most of the literature tend to show that earthworms are conducive to promoting mycorrhizal colonization of plants.

Table 1 The impact of the interaction between earthworms and AMF on the parameters of phytoremediation in heavy metal-contaminated soil

AMF species	Earthworms	Plants	Soil HMs	Effect of earthworms on AM colonization	Effect of AMF on phytoremediation parameters	Effect of Earthworms on phytoremediation parameters	Effect of AMF-Earthworms on phytoremediation parameters	Phytoremediation parameters	References
Glomus clarum	Eisenia andrei	Canavalia ensiformis	Cu	ns	++	+	+++	BCA _{Cu} of shoot	(Santana et al. 2019)
Funneliformis Mosseae	Eisenia Fetida	Zea mays L.	Zn	ns	+	+	+	Biomass of shoot	(Dehghanian et al. 2018)
			Fe		++	ns	+	C _{Zn} of shoot	
			Mn		+	+	+	C _{Fe} of shoot	
					+++	+	++	C _{Mn} of shoot	
Glomus intraradices and Glomus mosseae	Lumbricus rubellus L.	Zea mays L.	Cd	+	ns	+	ns	Available Cd	(Aghababaei et al. 2014a)
Acaulospora spp. and Glomus spp	Eisenia Fetida	Zea mays L.	As	ns	+	ns	ns	Biomass of root	(Hua et al. 2010)
Rhizophagus irregularis	Eisenia Fetida	Solanum nigrum L.	Cd	+	++	+	+++	C _{As} of root	(Wang et al. 2020)
					++	+	+++	Biomass of shoot	
					++	+	+++	BCA _{Cd} of shoot	
					+	ns	++	Available Cd	
Funneliformis Mosseae	Eisenia Fetida	Medicago sativa L.	Mo	+	+	+	++	Biomass of shoot	(Yang et al. 2024)
					+	++	+++	BCA _{Mo} of shoot	
					+	++	+++	Available Mo	
G. mosseae and G. intraradice	Pheretima sp.	Lolium multiflorum	Cd	+	-	+	+	Biomass of shoot	(Yu et al. 2005)
Glomus mosseae and Glomus intraradices	Pheretima Guillelmi	Leucaena leucocephala	Pb, Zn	+	++	+	+++	BCA _{Cd} of shoot	(Ma et al. 2006)
					+	ns	+	Biomass of plant	
					-	ns	-	BCA _{Pb} of root	
					-	ns	-	BCA _{Zn} of root	
					-	ns	-	Available Pb	
					-	ns	-	Available Zn	
Funneliformis mosseae and Septoglomus constrictum	Eisenia Fetida	Stachys inflata	Pb, Zn	-	++	+	+++	Biomass of plant	(Mahohi and Raiesi 2019)
					+	+	+++	BCA _{Pb} of plant	
					+	+	+++	BCA _{Zn} of plant	
Glomus intraradices and Glomus mosseae	Lumbricus rubellus L.	Zea mays L. and Helianthus annuus L.	Cd	ND	++	+	+++	Biomass of shoot	(Aghababaei and Raiesi 2015)
					--	-	--	C _{Cd} of shoot	

Note: The symbols “+” and “-” respectively represent the promotion and inhibition of biological inoculation treatments on the parameters of phytoremediation, and the number of “+” and “-” indicates the relative magnitude of the parameters. “ND” stands for no relevant data, and “ns” represents that the effect is not significant. “BCA” represents the bioaccumulation amount of heavy metals, and “C” represents the concentration of heavy metals

The impact of the interaction between earthworms and AMF on phytoremediation

Soil organisms act as essential regulators of biogeochemical cycles, mediating transformations of inorganic and organic nutrients while simultaneously sustaining soil structural integrity through organic matter dynamics and aggregate formation processes. Furthermore, they enhance plant productivity, mitigate pollutant-induced soil degradation, and maintain long-term soil fertility (Jeffries et al. 2003; Verbruggen et al. 2013). It has been shown that earthworms improve the availability of soil mineral nutrients under earthworms AMF plant systems. The enhancement of enzyme activity can effectively improve nutrient cycling and carbon accumulation in the rhizosphere and play a role in nutrient activation. After AMF colonizes the roots, a hyphal network is formed around the roots, playing a role in nutrient transfer (Milleret et al. 2009). Some literature has pointed out that there are negative effects among them (Tuffen et al. 2002). The reason for this phenomenon may be related to the nitrogen-phosphorus ratio in plants. In addition, soil microbial biomass (SMB) is an important indicator of soil biological activity and biochemical processes. High concentrations of heavy metals can suppress microbial activity, while earthworms and AMF can alleviate this negative effect. Earthworms mainly change soil pH, dissolved organic carbon (DOC) content and the structure of the microbial community through their own physical disturbance and the secretion of earthworm casts. AMF mainly enhance microbial activity by inducing plant root exudates and recruiting specific bacteria by themselves. Aghababaei et al. (2014a) found that after inoculating earthworms and AMF, the SMB would increase, and meanwhile the activities of soil urease, alkaline phosphatase (ALP), and soil respiration rate (SRR) all showed an increasing trend with the inoculation of earthworms and AMF.

Phytoremediation, using both earthworms and AMF together can enhance the effectiveness of the process by leveraging their unique functional advantages. However, variations in the types of earthworms, AMF, plant species, and soil environmental conditions can lead to different outcomes. While most studies suggest that these organisms have a synergistic effect, some research indicates they could inhibit each other's benefits in some cases (Table 1). Santana et al. (2019) found that in the soil contaminated with copper at a concentration of 100 mg kg^{-1} , the addition of AMF and earthworms increased the dry weight of the above-ground parts of sword beans by 81%, and at the same time increased the copper accumulation in the aboveground parts by 200%, enhancing the phytoextraction efficiency of sword beans. Dehghanian et al. (2018) found that in calcareous soil, the activities of earthworms and the inoculation of arbuscular

mycorrhizal fungi significantly decreased the soil pH value, increased DOC, microbial biomass carbon (MBC) and the available forms of Zn, Fe and Mn. Moreover, all the biological treatments led to a significant increase in the dry weight of the above-ground parts of maize as well as the concentrations of Zn, Fe and Mn. Aghababaei et al. (2014a) show that the addition of earthworms can increase the exchangeable cadmium in the soil, while the simultaneous addition of earthworms and AMF has no impact on this part of cadmium. Moreover, inoculating earthworms alone reduces the MBC but increases soil enzyme activity. On the contrary, inoculating AMF alone increases the MBC. The results indicate that in Cd-contaminated soil, the impact of using earthworms alone on the availability of cadmium is more important than that of AMF. Meanwhile, it also shows that the interactions among these organisms have a much more important impact on soil microorganisms than on the availability of Cd. Hua et al. (2010) shows that AMF inoculation leads to a significant increase in the AMF root colonization rate and root dry weight. Meanwhile, plants inoculated with both AMF and earthworms have higher arsenic concentrations in their roots, higher species diversity, a more balanced species composition, a more even species distribution and more food sources. Besides enhancing plants' absorption of pollutants, AMF and earthworms can also improve soil health by restoring the soil community structure. Wang et al. (2020) found that AMF and earthworms increased the accumulation of Cd in the tissues of *Solanum nigrum*. However, only AMF affected the distribution of Cd between the above-ground parts and roots. Moreover, both AMF and its combination with earthworms enhanced the plant availability of Cd by changing the chemical composition of Cd and reducing the pH value. In addition, in the soil with 120 mg kg^{-1} of Cd, the combined inoculation increased the removal amount of Cd by 149.3%.

Fig. 2 highlights the application of earthworms and AMF in phytoremediation of heavy metals in soil. The mucus and faeces secreted by them are rich in a large amount of humus and can also play a role in complexing heavy metals. Meanwhile, glomalin secreted by mycorrhizae, along with the structure of mycorrhizae themselves (such as arbuscules, vesicles, cell walls, etc.), can also sequester heavy metals. Additionally, mycorrhizae facilitate the formation of phytochelatins, which possess a strong complexing affinity for metals and encourage the compartmentalisation of metals in plant vacuoles, thereby reducing their toxicity (Ma et al. 2006). For phytoextraction, they also exhibit a synergistic effect. Firstly, the physical disturbance and secretions of earthworms can activate the mineral nutrients in the soil, change the soil pH value and increase the DOC, thus improving the availability of heavy metals. Secondly, the gut microorganisms of earthworms can also activate heavy

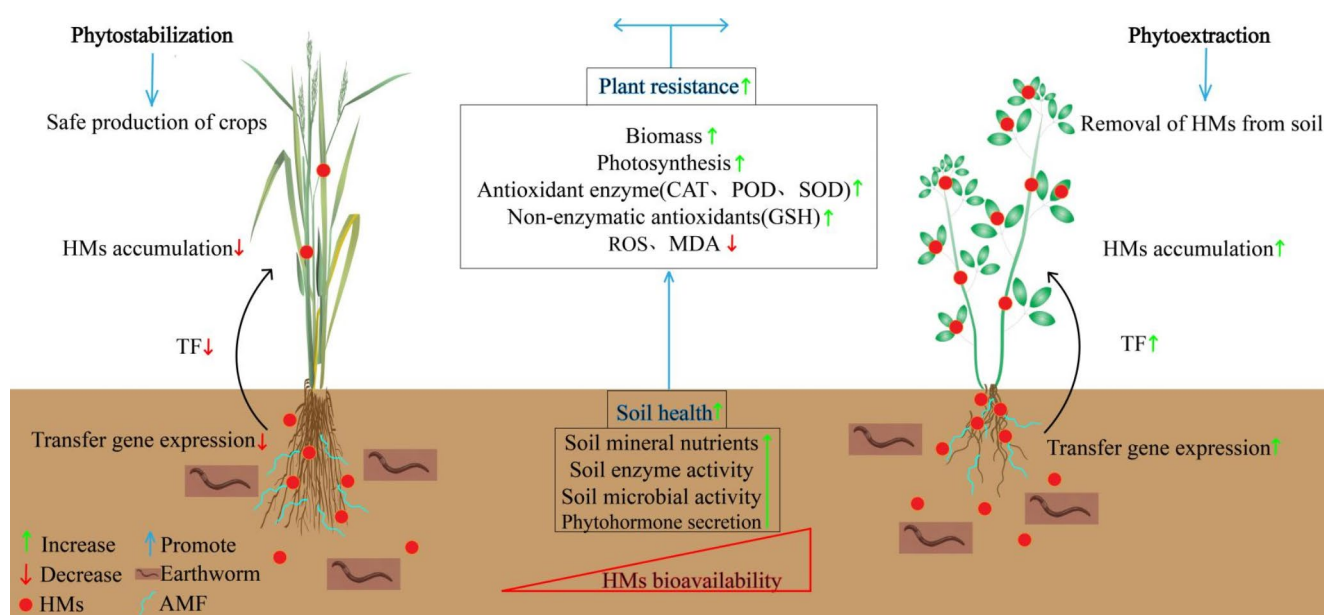


Fig. 2 The application of earthworms and AMF in phytoremediation of heavy metals in soil

metals in the soil and enhance plants' absorption of heavy metals. Meanwhile, mycorrhizae can regulate the expression of heavy metal-related transport genes, increase the plant transfer coefficient and promote the extraction of soil heavy metals by the above-ground parts of plants (Gonzalez-Guerrero et al. 2005; Yang et al. 2024) (Fig. 1).

The application of earthworms and AMF in phytostabilization and phytoextraction is highlighted in Fig. 2. For low heavy metal-polluted farmland, the combination of earthworms and AMF can help reduce heavy metal migration to crop grains, ensuring food safety and land productivity. In cases of moderate to severe pollution, hyperaccumulator plants with earthworm and AMF inoculation can enhance heavy metal extraction from the soil. A recent meta-analysis showed that AMF inoculation significantly decreased the accumulations of As and Cd in plant shoots (14.8% and 12.75%) and roots (20.59% and 3.58%). It also increased soil pH, organic carbon, and glomalin-related soil proteins while reducing available Cd by 2.35%, though it did not significantly affect available As and alkaline phosphatase levels (Tan et al. 2023). This provides important information on the great potential of AMF in phytostabilization. Thus, the action mechanisms of AMF and earthworms in phytoremediation are different. When these two organisms are combined, what is manifested is their complementary and additive effects, which depend on which organism plays a dominant role and are also related to soil and plant species. In our summary of some literature, it was found that earthworms and AMF predominantly exhibit a strong synergistic interaction in phytoextraction (Table 1), although their mechanisms remain to be further studied.

Conclusions and prospects

In phytoremediation, earthworms and AMF, as important auxiliary means, can greatly strengthen phytoremediation. Whether it is the phytoextraction of hyperaccumulator plants in highly heavy metal-polluted soil or the phytostabilization of farmland with moderate to low heavy metal pollution, they are both extremely promising bioremediation methods. From the perspective of resource utilization, since both of these organisms exist in nature, if they can be fully utilized, they can provide valuable resources for the future development of agriculture and the soil environment.

However, most of the current studies are pot experiments rather than field experiments. The soils used in the studies are mostly sterilized soils, which cannot simulate the real environment, and this is very likely to lead to different research results. In the future, a large number of field experiments are urgently needed to provide experimental data for the interaction between earthworms and AMF, which is a prerequisite for their wide application in actual contaminated soils. We recommend to focus on the following aspects in the future:

(1) Expansion of in-situ remediation of contaminated sites: Expand its application to a broader range of contaminated areas, including the in-situ remediation methods suitable for diverse contexts such as urban industrial legacy sites and farmlands affected by mining activities. By optimising operational techniques like inoculation and the release of combined methods, enhance the relevance and effectiveness of the remediation process. Optimize AMF-earthworm synergies through tailored inoculation and hybrid biological

methods. In-depth exploration of the combined enhancement effects and mechanisms of phytoremediation by AMF-earthworms. For example, the effects on metal dynamics vary with soil type, contaminant profile, and organism species. AMF may either enhance or inhibit metal uptake depending on plant-fungal symbiosis.

(2) Application in the context of climate change: Under global climate change and frequent extreme weather, explore the potential of the combined action of AMF-earthworms on enhancing the soil carbon sink function, such as promoting the sequestration of soil organic carbon, reducing greenhouse gas emissions, etc., to help the low-carbon and resilient development of agricultural ecosystems.

(3) Expansion of basic research: Conduct a more deep analyze of the complex signal transduction, material exchange and co-evolution mechanisms among arbuscular mycorrhizal fungi, earthworms, plant roots and other micro-organisms in the soil during the combined action, and reveal the profound interaction mysteries from the interdisciplinary perspectives of molecular biology, ecology and so on.

(4) Ecotoxicological research: Focus on the of pollutant toxicity responses and biomarker changes in the soil when these two are combined in specific polluted environments. High metal concentrations may inhibit earthworm and AMF activity; species selection must consider metal tolerance. This will provide a basis for a more scientific assessment of their ecological safety and the formulation of reasonable remediation and management strategies.

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Declarations

Competing interests The authors declare no competing interests.

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专利公告信息

发明专利证书

发明名称：一种高效降解土壤草甘膦的复合制剂及其使用方法和应用

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专利号：ZL 2022 1 1439347.7

授权公告号：CN 115990328 B

专利申请日：2022年11月17日

授权公告日：2024年07月02日

申请日时申请人：华南农业大学

申请日时发明人：杨旭;黎华寿;龚茂健;汤叶涛;仇荣亮

国家知识产权局依照中华人民共和国专利法进行审查，决定授予专利权，并予以公告。
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局长
申长雨

申长雨

2024年07月02日

证书号第8288829号



专利公告信息

发明专利证书

发明名称：一种动态研究植物根际环境变化的多层取样装置

专利权人：华南农业大学

地址：510000 广东省广州市天河区五山

发明人：杨旭;陈秋雨;姜彦岐;曾檬;李瑞婷;林庆祺;汤叶涛;仇荣亮

专利号：ZL 2024 1 1820826.2

授权公告号：CN 119774116 B

专利申请日：2024年12月11日

授权公告日：2025年09月23日

申请日时申请人：华南农业大学

申请日时发明人：杨旭;陈秋雨;姜彦岐;曾檬;李瑞婷;林庆祺;汤叶涛;仇荣亮

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局长
申长雨

申长雨

2025年09月23日



证书号第23161927号



专利公告信息

实用新型专利证书

实用新型名称：一种研究丛枝菌根真菌菌丝传递元素的装置

专利权人：华南农业大学

地址：510642 广东省广州市天河区五山483号

发明人：杨旭;蒋卓民;陈秋雨;崔团团;汤叶涛;仇荣亮

专利号：ZL 2024 2 2295707.1

授权公告号：CN 223176114 U

专利申请日：2024年09月19日

授权公告日：2025年08月01日

申请日时申请人：华南农业大学

申请日时发明人：杨旭;蒋卓民;陈秋雨;崔团团;汤叶涛;仇荣亮

国家知识产权局依照中华人民共和国专利法进行审查，决定授予专利权，并予以公告。

专利权自授权公告之日起生效。专利权有效性及专利权人变更等法律信息以专利登记簿记载为准。

局长
申长雨

申长雨

2025年08月01日



合同编号:

技术转让（专利权）合同

项目名称: 1. 一株高效降解丁草胺的变栖克雷伯氏菌 WX-01 及其应用; 2. 一种高效降解土壤草甘膦的复合制剂及其使用

受让方（甲方）: 广东大海生物科技有限公司

让与方（乙方）: 华南农业大学

签订时间: 2024. 12. 5

签订地点: 华南农业大学

有效期限: 长期

中华人民共和国科学技术部印制

填 写 说 明

一、本合同为中华人民共和国科学技术部印制的技术转让（专利权）合同示范文本，各技术合同认定登记机构可推介技术合同当事人参照使用。

二、本合同书适用于一方当事人（让与方、原专利权人）将其发明创造专利权转让受让方，受让方支付约定价款而订立的合同。

三、签约一方为多个当事人的，可按各自在合同关系中的作用等，在“委托方”、“受托方”项下（增页）分别排列为共同受让人或共同让与人。

四、本合同书未尽事项，可由当事人附页另行约定，并作为本合同的组成部分。

五、当事人使用本合同书时约定无需填写的条款，应在该条款处注明“无”等字样。

技术转让（专利权）合同

受让方（甲方）： 广东大海生物科技有限公司

住 所 地： 深圳市宝安区松岗街道潭头社区芙蓉路 21 号 442

法定代表人： 刘付凡芳

项目联系人： 刘付凡芳

联系方式： 135 3009 3201

通讯地址： 江门市新会区古井镇南朗加油站南 100 米南荣渔药店
后面（大海一号）

电话： 135 3009 3201 传真： 无

电子信箱： 821118376@qq.com

让与方（乙方）： 华南农业大学

住 所 地： 广州天河五山 华南农业大学

法定代表人： 薛红卫

项目联系人： 黎华寿

联系方式：

通讯地址： 广州天河五山 华南农业大学资源环境学院

电话： 13610070181 传真：

电子信箱： lihuashou@scau.edu.cn

本合同乙方将其 1.一株高效降解丁草胺的变栖克雷伯氏菌
WX-01 及其应用；2 一种高效降解土壤草甘膦的复合制剂及其使用 的
专利权转让甲方，甲方受让并支付相应的转让价款。双方经过平等协

商，在真实、充分地表达各自意愿的基础上，根据《中华人民共和国民法典》的规定，达成如下协议，并由双方共同恪守。

第一条：本合同转让的专利权：

1. 为发明（发明、实用新型、外观设计）专利。
2. 发明人/设计人为：（1）黎华寿，魏鑫，彭雅琴，刘晓玉；（2）杨旭，黎华寿，龚茂健，汤叶涛，仇荣亮。
3. 专利权人：华南农业大学。
4. 专利授权日：2021-03-19 ;2023-04-21。
5. 专利号：ZL 202011595044.5; ZL 202211439347.7。
6. 专利有效期限：长期。
7. 专利年费已交至2024.12.30。

第二条：乙方在本合同签署前实施或许可本项专利权的状况如下：

1. 乙方实施本项专利权的状况（时间、地点、方式和规模）：乙方目前并未实施使用。
2. 乙方许可他人使用本项专利权的状况（时间、地点、方式和规模）：乙方并无许可他人使用。
3. 本合同生效后，乙方有义务在5日内将本项专利权转让的状况告知被许可使用本发明创造的当事人。

第三条：本合同生效后办理专利转让手续前乙方不得再使用本项目专利。

第四条：为保证甲方有效拥有本项专利权，乙方应向甲方提交以下技术资料：

1. 专利权证书复印件；

2. 专利技术资料。

第五条：乙方向甲方提交技术资料的时间、地点、方式如下：

1. 提交时间：合同签订后七个工作日内

2. 提交地点：广州

3. 提交方式：现场交接或电子交接

第六条：本合同签署后，由甲方负责在15日内办理专利权转让登记事宜。

第七条：乙方对本合同生效后专利权被宣告无效，不承担法律责任。

第八条：甲方向乙方支付该项专利权转让的价款及支付方式如下：

1. 专利权的转让价款总额为：40000 元（人民币）

2. 专利权的转让价款由甲方一次（一次、分期或提成）支付乙方。

（1）具体支付方式和时间如下：自双方签章日起七个工作日内，甲方向乙方一次性支付专利转让费 40000 元（人民币）

乙方开户银行名称、地址和帐号为：华南农业大学

开户银行：中国工商银行广州分行五山支行

地址：广东省广州市天河区五山路 483 号

帐号：3602002609000310520

第九条：双方确定：

1. 甲方有权利用乙方转让专利权涉及的发明创造进行后续改进。

由此产生的具有实质性或创造性技术进步特征的新的技术成果，归甲方所有。

2. 乙方有权在已交付甲方该项专利权后，对该项专利权涉及的发明创造进行后续改进。由此产生的具有实质性或创造性技术进步特征的新的技术成果，归乙方所有。

第十条：双方确定，如一方违约，应当按照法律向其余一方承担违约责任。

第十一条：双方确定，在本合同有效期内，甲方指定刘付凡芳为甲方项目联系人，乙方指定黎华寿为乙方项目联系人。项目联系人承担以下责任：

1. 协助甲方共同办理此项专利权转让登记事宜；
2. 向甲方提交专利转让相关所需资料。

一方变更项目联系人的，应当及时以书面形式通知另一方。未及时通知并影响本合同履行或造成损失的，应承担相应的责任。

第十二条：双方确定，出现下列情形，致使本合同的履行成为不必要或不可能的，可以解除本合同：

1. 因发生不可抗力；

第十三条：双方因履行本合同而发生的争议，应协商、调解解决。协商、调解不成的，确定按以下第1种方式处理：

1. 提交广州市仲裁委员会仲裁；
2. 依法向人民法院起诉。

第十四条：双方约定本合同其他相关事项为：无。

第十五条：本合同一式肆份，具有同等法律效力。

第十六条：本合同自国家专利行政主管部门登记之日起生效。

甲方：广东大海生物科技有限公司 (盖章)

法定代表人 / 委托代理人：刘付凡芳 (签名)

年 月 日

乙方：薛红已 (盖章)

法定代表人 / 委托代理人：薛红已 (签名)

年 月 日

印花税票粘贴处：

（此页由技术合同登记机构填写）

合同登记编号：

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1. 申请登记人：_____

2. 登记材料：（1）_____

（2）_____

（3）_____

3. 合同类型：_____

4. 合同交易额：_____

5. 技术交易额：_____

技术合同登记机构（印章）

经办人：

年 月 日

2024年华南农业大学大学生创新创业训练计划项目

结题证书

项目编号：2024105641214

项目级别：校级项目类型：创新训练项目

项目名称：龙葵种质资源的筛选及龙葵富集镉的机制研究

项目主持人：朱郴指导老师：杨旭

项目组成员：刘禹辰、曾渚民、张铭轩、姚秀文

该项目已完成，经专家组评审准予结题，验收结果为合格，特发此证。

华南农业大学创新创业学院
二〇二五年十一月

创新创业学院

第四届全国农业资源与环境专业
大学生实践技能竞赛

获奖证书

作品名称：广东省恩平市马铃薯试验田土壤现状
及优化施肥方案

完成单位：华南农业大学

团队成员：曾渚民、朱郴、余秋涛

指导老师：伏广农、杨旭

获奖等级：优胜奖

国务院学位委员会第八届农业资源与环境学科评议组秘书处
全国农业资源与环境专业大学生实践技能竞赛组委会
华南农业大学资源环境学院（代章）

二〇二四年十一月