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丛枝菌根真菌对溶解态和胶体态Cd淋溶的影响

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摘 要:采用粒径0.2 mm的石英砂制备30 cm高的砂柱,在接种和不接种丛枝菌根真菌(Arbuscular mycorrhizal fungi, AMF)条件下种植玉米,施用1 mg·L^{-1}的溶解态和胶体态Cd,开展砂柱培养和模拟淋溶试验,研究AMF对砂粒比表面积、砂柱溶液Cd浓度和淋溶流失的影响。结果表明:AMF菌丝在砂柱中生长和分泌球囊霉素相关土壤蛋白(GRSP),增大石英砂的比表面积,增加石英砂对Cd的吸附量与吸附能力;接种AMF显著降低砂柱10 cm处溶液中溶解态和胶体态Cd的浓度,减少Cd的淋溶流失量;相关性分析表明,在AMF接种情形下,砂柱中GRSP含量与砂粒Cd吸附量呈显著正相关,GRSP含量与砂柱溶液Cd浓度呈显著负相关,且砂粒Cd吸附量与砂柱溶液Cd浓度呈显著负相关,砂柱20 cm深度的砂粒Cd吸附量与Cd流失量呈显著负相关,砂柱30 cm深度的溶液Cd浓度与Cd流失量呈显著正相关。可见,AMF菌丝通过分泌GRSP、增大石英砂的比表面积,增强石英砂对Cd的吸附,从而降低砂柱溶液Cd浓度与Cd淋溶流失。

关键词:丛枝菌根真菌;球囊霉素相关土壤蛋白;比表面积;Cd吸附;淋溶流失

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Effects of arbuscular mycorrhizal fungi on leaching of dissolved and colloidal Cd

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Abstract: In the present study, a sand column at a height of 30 cm was made by quartz sand with a diameter of 0.2 mm. Maize(*Zea mays* L.) was cultivated with or without an inoculation of arbuscular mycorrhizal fungi (AMF). Dissolved and colloidal Cd of 1 mg·L^{-1} were added into the sand column. Then, sand column cultivation and leaching experiments were carried out to investigate the effects of AMF on the specific surface area of the quartz sand, Cd concentration in the sand column solution, and Cd leaching loss. The results showed that: AMF mycelium grown in the sand column and secreted glomalin-related soil protein (GRSP) increased the specific surface area of quartz sand and promoted the sorption amount and adsorption capacity of the quartz sand for Cd; the inoculated AMF significantly reduced the concentration of dissolved and colloidal Cd in the solution at a depth of 10 cm in the sand column and reduced the Cd leaching loss; and the correlation analysis indicated that the GRSP content in the sand column had a significant positive correlation with the Cd adsorption amount of the quartz sand but was negatively correlated with the Cd concentration of solutions in the sand column for the AMF inoculation treatment. Furthermore, the Cd adsorption amount of the quartz sand had a significant negative correlation with the Cd concentration of solutions in the sand column. The Cd adsorption amount of the quartz sand at a 20 cm depth was significantly negatively correlated, while

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the Cd concentration of the solution at a 30 cm depth was significantly positively correlated with the Cd leaching loss from the sand column. Thus, the AMF mycelium secreted GRSP increase the specific surface area of the quartz sand and its Cd adsorption and thereby, reduce the Cd concentration and leaching loss from the sand column.

Keywords: arbuscular mycorrhizal fungi; glomalin-related soil protein; specific surface area; cadmium adsorption; leaching loss

采矿、施肥和污灌等活动导致我国许多农田出现不同程度的Cd污染[1]。根据《全国污染土壤调查公报》,Cd的点位超标率为7.0%,为重金属之首[2]。Cd在土壤中具有毒性、持久性和环境迁移性,在表层累积会导致土壤肥力降低和农作物减产[3-4]。由于降雨或灌溉作用造成的地表径流和淋溶,使积累在土壤表层的Cd会从上向下迁移进而造成Cd淋溶流失和污染扩散,污染深层土壤和地下水[5]。淋溶迁移的Cd主要以溶解态、胶体态和悬浮态等形式存在。溶解态Cd存在于溶液中,是吸附反应的直接来源,其中Cd^{2+}是主要赋存形式[6],进入胶体后和胶体共迁移[7]。

Cd淋溶的影响因素很多,其中微生物对Cd的迁移就有至关重要的影响。丛枝菌根真菌(Arbuscular mycorrhizal fungi,AMF)在土壤中广泛存在,能与陆地上80%的维管植物形成共生关系[8-9]。AMF能促进植物吸收养分并减少重金属毒害,同时可改善土壤性质和减少土壤养分流失[10-12]。AMF在土壤中生长形成密集的菌丝网络,菌丝缠绕住土壤颗粒[13],且菌丝上有结合位点可以吸附固定Cd,减少Cd的流失[14];菌丝衰亡分解后会释放多糖、蛋白质等,这些有机组分被吸附后可改变土壤颗粒的表面化学性质。此外,AMF释放的球囊霉素相关土壤蛋白(Glomalin-related soil protein,GRSP)是能促进土壤胶结、提高土壤团聚体稳定性的生物胶[15],也能结合和稳定Cd离子[16],增强土壤对Cd的吸附能力[17]。Wang等[18]在对沿海周围土壤的研究中发现GRSP给重金属提供了孔隙和内表面,促进土壤吸附重金属,且GRSP固着的重金属量远远大于重金属释放量,对水质改善起着重要作用。然而,AMF对Cd淋溶的作用机制还需要深入探讨。

土壤对Cd有一定的吸附能力,但是土壤情况复杂,土壤任何性质和组分的变化都会对Cd吸附解吸和迁移有明显的影响[19],石英砂成分简单,可减少土壤中的干扰。本试验用石英砂作培养基质,添加溶解态和胶体态Cd溶液,在模拟降雨条件下研究接种AMF对Cd淋溶流失量和砂柱溶液Cd浓度的影响以及AMF菌丝对砂粒表面性质与Cd吸附-解吸特征的影响,丰富生物因素对土壤Cd淋溶流失的影响及机理方面的理论与认识。

1 材料与方法

1.1 供试材料

石英砂(主要成分SiO_2),粒径为0.2 mm,用2%稀硝酸浸泡后用蒸馏水洗净,在121 ℃下灭菌2 h。

玉米(*Zea mays* L.),品种为会单四号,种子用10%次氯酸钠(浸泡2 min)和75%乙醇(浸泡1 min)进行表面消毒后置于28 ℃恒温培养箱中催芽3 d,露白后播种。

摩西管柄囊霉(*Funneliformis mosseae*),由北京市农林科学院植物营养与资源研究所提供菌种(编号为BGC YN05 1511C0001BGCAM0013),经实验室扩繁后获得菌剂。

1.2 溶解态和胶体态Cd的制备

溶解态Cd配制:设置Cd离子的浓度为1 mg·L^{-1}。称取$CdCl_2·2.5H_2O$(分析纯)10.2 mg溶于1 L蒸馏水中配制成溶解态Cd浓度为1 mg·L^{-1}的溶液。

胶体态Cd制备:按徐伟慧等[20]的方法,制备清洁胶体储备液备用。取50 mL清洁胶体储备液和50 mL浓度为50 mg·L^{-1}的$CdCl_2$溶液混合,往复振荡24 h,4 000 r·min^{-1}离心10 min,将上清液吸出,往离心管中加入50 mL蒸馏水,超声分散30 min,重新制备得胶体悬浊液,重复2次。该溶液即为试验用的胶体态Cd浓度为100 mg·L^{-1}吸附Cd的土壤胶体溶液,试验时再稀释为1 mg·kg^{-1}胶体态Cd溶液[21]。

1.3 砂柱制备与淋溶试验

制作高30 cm、直径11 cm、顶端敞口的PVC管,在管的底部铺设尼龙布,并分别在10、20、30 cm处设砂柱溶液取样口,并安装排水龙头。石英砂灭菌后采用湿法[22]填入管中,砂柱高度30 cm。设置对照(CK)和接种AMF(AMF)两个处理,每个处理8个平行。在AMF处理砂柱表层铺2 cm的菌种,种植玉米后再覆盖3 cm的石英砂,对照则用灭菌石英砂装填。每管种植3株玉米,每隔3 d每管砂柱浇Hoagland′s营养液100 mL和去离子水200 mL。培养30 d后,各处理取4个平行加入溶解态Cd,另外4个平行加入胶体态Cd溶液,培养7 d后用淋溶装置淋溶,每管淋溶去离

子水6 L。采用Rhizons提取器收集10、20、30 cm处砂柱溶液，管底部用塑料瓶收集淋溶液。

1.4 AMF相关指标测定

淋溶结束后收获玉米，测定AMF相关指标：酸性品红法测定菌根侵染率[23]；按照Thapa等[24]的方法测定菌丝密度；采用湿筛-倾析法测定孢子数[25]；考马斯亮蓝比色法测定球囊霉素土壤相关蛋白浓度[26]。

1.5 溶液Cd浓度测定与流失量计算

砂柱溶液和淋溶液收集于塑料瓶内保存，各取25 mL采用HNO_3-H_2O_2消解-石墨炉原子吸收光谱法测定Cd浓度。

从淋溶管底部采集的淋溶液，测量淋溶液体积和Cd浓度，由流失量=淋溶液Cd浓度×淋溶液体积，得出石英砂柱Cd淋溶流失量。

1.6 砂粒比表面积测定

淋溶结束后收集石英砂，将石英砂风干后采用BET法测定石英砂的比表面积[27]。

1.7 砂粒吸附量与吸附能力测定

砂粒吸附量测定：称取1 g砂柱各层的样品于50 mL塑料离心管中，加入0.1 mol·L^{-1}的$NaNO_3$溶液25 mL，解吸2 h，在恒温振荡箱（25 ℃）中于180 r·min^{-1}的速度高速振荡2 h后，恒温静置过夜，4 000 r·min^{-1}的速度高速离心10 min，取上清液过滤，用原子吸收光谱法测定金属离子Cd的浓度即石英砂吸附量。平衡时单位石英砂解吸溶液中重金属的量计算式为：

$$Q_d = \frac{C_d \times V}{m}$$

式中：Q_d为解吸平衡时单位石英砂解吸的溶液中重金属的量，mg·g^{-1}；C_d为解吸平衡时溶液的重金属离子的质量浓度，mg·L^{-1}；V为解吸溶液的体积，L；m为石英砂烘干质量，g。

砂粒吸附能力测定：称取石英砂和含AMF石英砂1 g于50 mL的离心管中，加入1 mg·L^{-1}的Cd^{2+}溶液，置于恒温（25 ℃）振荡箱中以180 r·min^{-1}速度离心振荡2 h，静置过夜，再以4 000 r·min^{-1}的速度高速离心10 min，取上清液过滤，用石墨炉测定Cd^{2+}，每个处理3次重复[27]。用差减法计算石英砂对Cd^{2+}的吸附量，即砂粒的吸附能力。

1.8 数据处理与统计分析

试验数据采用Excel整理，采用OriginPro 8进行图表绘制。采用SPSS 23.0对数据进行统计分析和整理，独立样本使用T检验进行显著性分析，并对各数据进行Pearson相关性分析。

2 结果与分析

2.1 AMF生长指标与砂粒比表面积

如表1所示，接种AMF处理玉米根系的AM真菌侵染率为30%左右，菌丝密度为30 cm·g^{-1}左右，孢子数为12个·g^{-1}左右，添加Cd的形态对此没有影响。石英砂的比表面积见表2，在添加溶解态Cd条件下，对照为0.019 m^2·g^{-1}，接种处理为0.119 m^2·g^{-1}；在添加胶体态Cd条件下，对照为0.076 m^2·g^{-1}，接种处理为0.336 m^2·g^{-1}。可见，接种AMF增加了石英砂的比表面积。

表1 AMF生长指标

Table 1 AMF growth indicator

Cd形态 Cd forms	根系侵染率 Mycorrhizal colonization/%	菌丝密度 Hyphae density/(cm·g^{-1})	孢子数 Spore count/(个·g^{-1})
溶解态	30.02±1.76	29.94±1.12	12.25±2.95
胶体态	31.56±1.28	31.27±0.85	12.25±1.09

表2 石英砂粒比表面积

Table 2 Specific surface area of quartz sand grains

Cd形态 Cd forms	处理 Treatments	比表面积 Specific surface area/(m^2·g^{-1})
溶解态	CK	0.019
	AMF	0.119
胶体态	CK	0.076
	AMF	0.336

2.2 接种AMF砂柱中GRSP含量

如图1所示，溶解态Cd处理下，砂柱中10、20、30 cm处的GRSP含量分别为17.09、20.13、18.95 g·kg^{-1}，胶体态Cd处理下，砂柱中10、20、30 cm处的GRSP含量分别为18.04、21.53、20.24 g·kg^{-1}，Cd的形态对GRSP的含量无显著影响。

2.3 砂粒Cd吸附量与吸附能力

添加溶解态Cd，接种处理在10、20 cm处极显著增加石英砂的Cd吸附量，均增加了55%；在30 cm处吸附量显著增加40%。添加胶体态Cd，接种处理在10、20 cm处极显著增加石英砂的Cd吸附量，分别增加了75%和44%；在30 cm处吸附量增加不显著。由此可知，接种AMF能增强石英砂对Cd的吸附能力。总体来看，石英砂对溶解态Cd的吸附能力为0.528 mg·kg^{-1}，接种AMF可提高至0.756 mg·kg^{-1}，增幅为30%；石英砂对胶体态Cd的吸附能力为0.600 mg·kg^{-1}，接种AMF可提高至0.790 mg·kg^{-1}，增幅为24%。

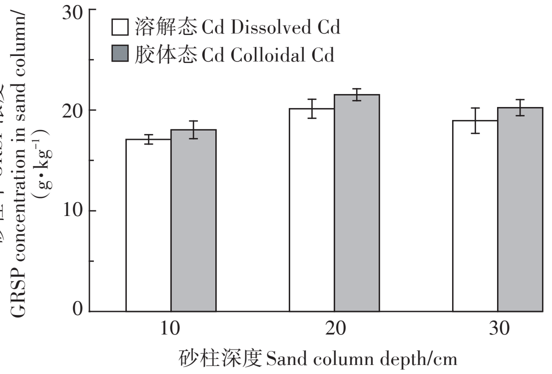
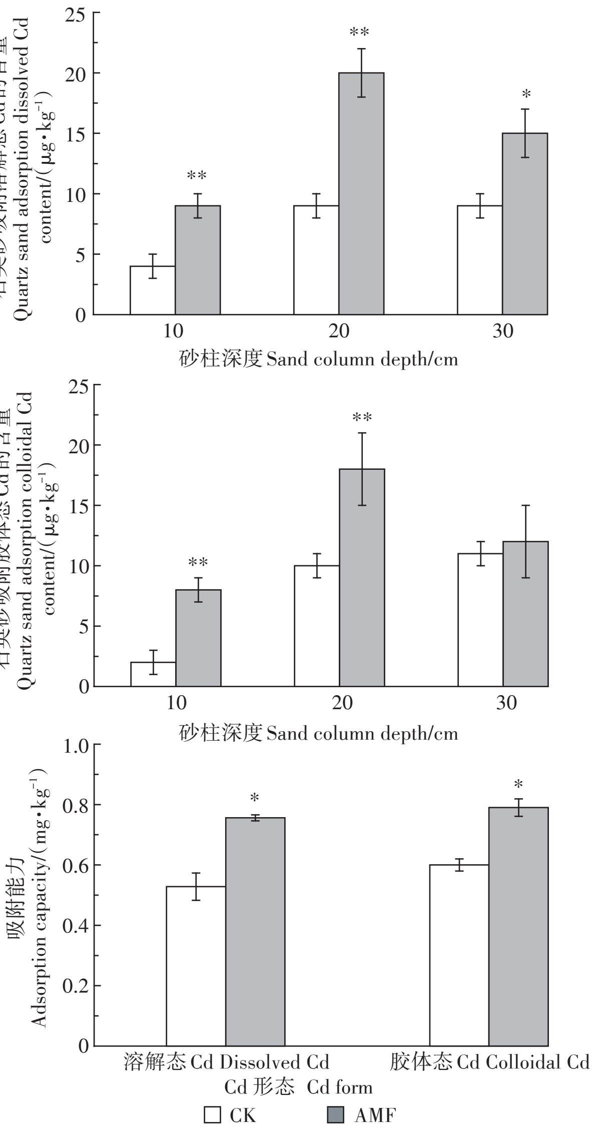


图1 接种AMF砂柱中GRSP含量

Figure 1 GRSP content in AMF sand column



*、**分别表示在5%、1%的水平上差异显著。下同

The *, ** indicate significant levels at 5% and 1% level respectively. The same below

图2 石英砂对溶解态和胶体态Cd的吸附量和吸附能力

Figure 2 Sorption amount and adsorption capacity of dissolved and colloidal Cd by quartz sand

接种AMF后石英砂的吸附量随砂柱深度呈先增后减的趋势,在20 cm处吸附量最大。

2.4 砂柱溶液Cd浓度和流失量

添加溶解态Cd,接种AMF处理10 cm处砂柱溶液的Cd浓度显著减少了25%;添加胶体态Cd,接种AMF处理10 cm处砂柱溶液Cd浓度极显著减少了45%。由此可知,接种AMF能显著降低砂柱表层溶液中的Cd浓度。从流失量来看,胶体态Cd小于溶解态Cd。其中,添加溶解态Cd条件下接种AMF处理Cd的流失量极显著降低35%,添加胶体态Cd条件下接种AMF处理Cd的流失量显著降低13%。

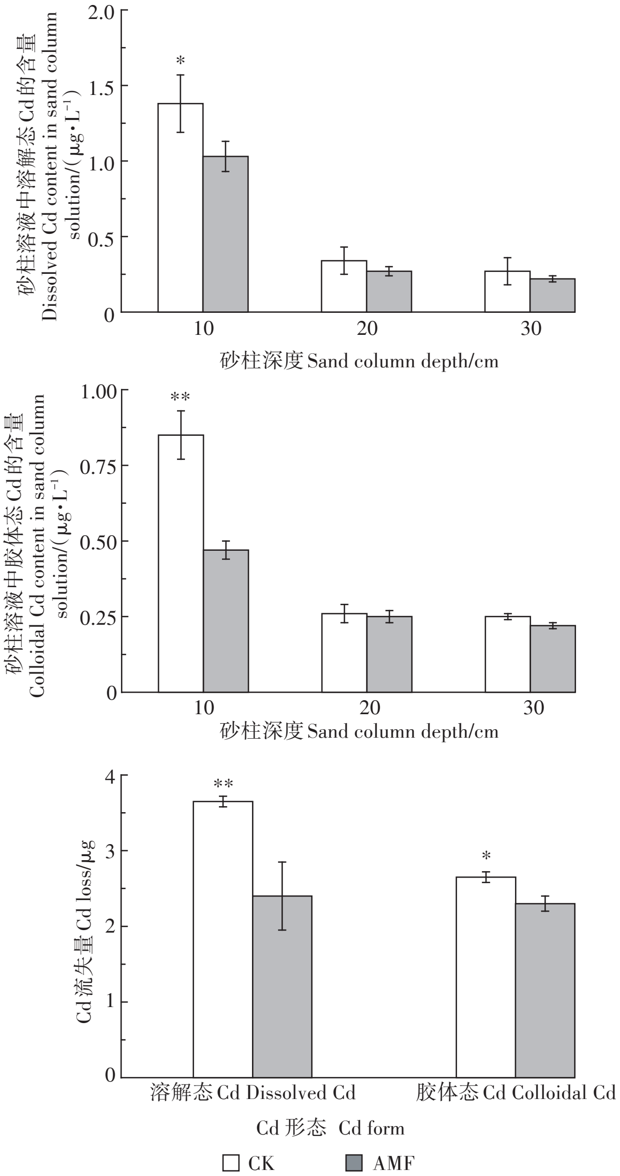


图3 砂柱溶液中溶解态和胶体态Cd浓度和流失量

Figure 3 Concentrations and loss amount of dissolved and colloidal Cd in sand column solution

2.5 相关性分析

相关分析表明，AMF接种情形下，砂柱3个深度的GRSP含量与砂粒Cd吸附量（r=0.685，P<0.05，n=24）呈显著正相关，与砂柱溶液Cd浓度（r=−0.434，P<0.05，n=24）呈显著负相关；且砂粒Cd吸附量与砂柱溶液Cd浓度（r=−0.407，P<0.05，n=24）呈显著负相关。

如表3所示，在接种AMF处理下，砂柱20 cm深度的砂粒Cd吸附量与Cd流失量呈显著负相关，砂柱30 cm深度的溶液Cd浓度与Cd流失量呈显著正相关。表明接种AMF增加砂粒对Cd的吸附，有助于降低砂柱溶液Cd浓度和Cd的淋溶流失。

表3 砂柱Cd流失量与砂粒Cd吸附量、溶液Cd浓度的相关性分析

Table 3 Correlation analysis of Cd loss in sand column with Cd adsorption of sand particles and concentration of solution Cd

砂柱因子 Sand column factor	砂柱深度 Sand column depth/cm		
	10	20	30
砂粒Cd吸附量	0.323	−0.774*	−0.324
砂柱溶液Cd浓度	−0.231	−0.081	0.825*

注：*表示P<0.05，n=8。
Note：* means P<0.05，n=8.

3 讨论

试验通过接种AMF增大石英砂吸附量，降低砂柱溶液中Cd浓度，减少Cd的流失量。AMF因减少土壤养分流失的生态功能而备受关注，AMF的菌丝网络吸收土壤养分并传输给寄主植物，使得土壤在高降雨强度下减少了40%的氮流失和50%磷流失[28]。Heijden等[29]对3种植物进行AMF的接种，结果发现无论种植哪一种植物，AMF都降低了渗滤液的体积。He等[30]研究强降雨下AMF对Cd淋溶的影响发现AMF使Cd污染土壤中T-GRSP含量和大团聚体比例增加，降低了Cd污染土壤中Cd的回流和淋滤浓度，与本试验中接种AMF减少Cd的浓度和流失量的研究结果一致。此外菌丝上的结合位点将Cd吸附固定在植物的根部[31]，也能减少Cd在溶液中的浓度。本试验相关性分析中，接种AMF处理中砂柱20 cm深度的砂粒Cd吸附量与Cd流失量呈显著负相关，砂柱30 cm深度的溶液Cd浓度与Cd流失量呈显著正相关。由此可见，AMF有降低Cd淋溶流失的能力。

有研究表明，AMF的分泌物有吸附Cd的作用，从而降低溶液中的Cd浓度。GRSP由专性生物营养型AMF的活菌丝产生[32]，具有胶状、非水溶性和高比表面积的特性，GRSP黏合土壤颗粒，增加土壤中的微孔量使土壤的表面积增大[18,33]，这可能是石英砂比表面积增大的原因，AMF对土壤理化性质的影响已被大量报道，但对石英砂比表面积等物理性质影响的相关报道目前较少，需要大量试验验证。本试验相关性分析表明，砂柱中GRSP含量与砂粒Cd吸附量呈显著正相关，与砂柱溶液Cd浓度呈显著正相关，说明了GRSP可能增大石英砂吸附量，降低砂柱溶液中的Cd浓度，从而减少Cd的流失。Wang等[18]用GRSP去除水中的Cd、Cu试验中发现，GRSP能有效吸附76%的Cd和95%的Cu，证实了GRSP对Cd的吸附作用。GRSP吸附土壤中重金属主要是因为GRSP上的羰基、羧基、羟基与重金属有很高的配位性[34]，Cd与这些官能团形成配体后沉积在土壤中，降低了溶液中Cd浓度[35]，也有学者发现GRSP上基团也能吸附土壤中的一些阳离子，和土壤中的重金属形成竞争关系，影响GRSP对Cd的吸附[36]。此外，AMF能分泌低分子量有机酸（LMWOAs），促进重金属的螯合和吸附，在土壤重金属的固定中发挥着重要作用[37]。综上所述，AMF分泌的GRSP对Cd淋溶流失的减少有一定的贡献，但AMF分泌物对重金属吸附的机理还不完善，应做深入研究。

不同形态Cd的迁移能力不一样。本试验中胶体态Cd的流失量比溶解态Cd的流失量小，主要原因是胶体态Cd是土壤胶体吸附Cd^{2+}，胶体的高表面积和反应活性使其能够吸附重金属，同时胶体通过在基质毛细管中的阻滞作用和在大孔壁上的共沉积作用，阻碍Cd在砂柱中的迁移[38-39]；但也有学者认为胶体的存在会促进Cd迁移[40]。不同形态Cd处理下，AMF也会有不同的反应机制。在溶解态Cd处理下，Cd^{2+}优先与带负电荷的菌丝和根表面成分结合，AMF菌丝的分泌物将溶解态Cd由离子态向残渣态转变，并沉淀在砂柱中[36-37]；关于AMF富集胶体态Cd的报道较少，但有学者发现AMF会释放羧酸溶解碳酸盐岩和氧化物结合的重金属，从较难溶解的形态中溶解重金属[41]。有的微生物对溶解态Cd的富集能力比胶体态Cd强，因为与胶体结合的Cd不能被微生物充分捕捉[42]。AMF对不同形态Cd响应机制还要深入研究。

4 结论

（1）接种AMF能增大石英砂比表面积，增加石英砂对Cd的吸附能力，减少砂柱溶液中Cd浓度，降低Cd的淋溶流失量。

(2)不同形态的Cd对AMF的生长和GRSP的含量没有明显的影响,但试验中胶体态Cd的流失量小于溶解态Cd的流失量。

(3)接种AMF砂柱中GRSP浓度与石英砂Cd吸附量呈显著正相关,与砂柱溶液Cd浓度呈显著负相关,石英砂Cd吸附量与砂柱溶液Cd浓度呈显著负相关;且砂柱20 cm处的砂粒Cd吸附量与Cd流失量呈显著负相关,30 cm处的溶液Cd浓度与Cd流失量呈显著正相关。综上所述AMF菌丝及其分泌的GRSP对减少Cd的淋溶流失有一定的贡献。

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