The Effects of Photosynthetic Bacteria and Arbuscular Mycorrhizal Fungi on Phytoremediation for Soils Contaminated by Heavy Metals

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Introduction

Introduction (1/4)

- Phytoremediation is a environmentally friendly ecotechnology, which uses plants to clean up contaminated soils and ground water.
- The plants can help to break down or degrade organic pollutants or to stabilize metal contaminants by acting as filters or traps.
- Thus, how to achieve fast plants growth and high biomass yield in order to let plant uptake more heavy metals from contaminated soils into the plant tissue is worth to study further.

Introduction (2/4)

- Photosynthetic bacteria (PSB) were found able to enhance productivity of plant hormones, such as auxin, kinetin and zeatin (Serdyuk, Smolygina *et al.*, 1993).
- Auxin has been proved able to stimulate and increase the growth of plants (Vanderhoff and Stahl, 1975).
- So, the first purpose of this study is to investigate the effects of photosynthetic bacteria (PSB) on uptake of Cd in the three plant species.

Introduction (3/4)

- In addition, heavy metal uptake and tolerance for plants were found depending on their symbiotic mycorrhizal fungi.
- Bradley *et al.* (1981) first found that mycorrhizal fungi could reduce the uptake of Cu and Zn in the plant species of *Calluna valgaris*., while mycorrhizal fungi were found in plant roots growing on heavy metal contaminated sites (Shetty, Hetrick *et al.*, 1995), indicating their tolerance to heavy metal.
- Presently, more researchers are studying the interactions between mycorrhizal fungi and heavy metals. (Poster Session 1-#35-37

Introduction (4/4)

- The second purpose of this study is to investigate the effects of arbuscular mycorrhizal fungi (AMF) on metal uptake by different plant species.

Materials and Methods

PSB Tests

PSB Tests (1/3)

- Three plant species, allegator weed (*Alternanthera philoxeroides*), vetiveria (*Vetiveria zizanioides*), and spider brake fern (*Pteris multifida*), [not Chinese brake fern (*Pteris vittata*)], were used in the study.

As and Cd (Mei Lei *et al.*, 2008)

chilopod grass

allegator weed

vetiveria

spider brake fern

PSB Tests (2/3)

- The clean soil samples were mixed in a large container and dried at room temperature for two weeks.
- After that, the soil was crushed and sieved through a 2-mm mesh to remove stones and plant debris.
- The Cd contaminated soil (10 mg/kg) was prepared artificially by adding $Cd(NO_3)_2 \cdot 4H_2O$, and then put in the pots and mixed by stirrer for 10 minutes.
- After two-week equilibrium, the Cd contaminated pots were then transplanted with the three plant species selected, meanwhile PSB (mix cultures of ***Rhodospseudomonas palustris***, ***Rhodospirillium sphaeroides***, ***Rhodobactor capsulatus***, and ***Rhodospirillium rubrum***) was added into the soil.

PSB Tests (3/3)

- The tests were run in a greenhouse, and under a condition in a 2×2 factorial completely randomised design, with two replicates.
- The pot tests with the three plant species were controlled either with or without addition of PSB.
- In the 8th week, the plant in each pot was carefully removed from the pots.
- Each harvested plant was washed by deionized water and separated into aboveground part (leaves and stem) and belowground part (roots including rhizomes) for analysis of Cd concentrations, meanwhile soil in each pot was also tested for Cd.




AMF Tests

AMF Tests (1/3)

- Two local plant species, hairy beggarticks, or devil's needles (*Bidens pilosa*) and stinking passion flower, or bush passionfruit (*Passiflora foetida*), were used in the second part of this study.

devil’s needles

stinking passion flower

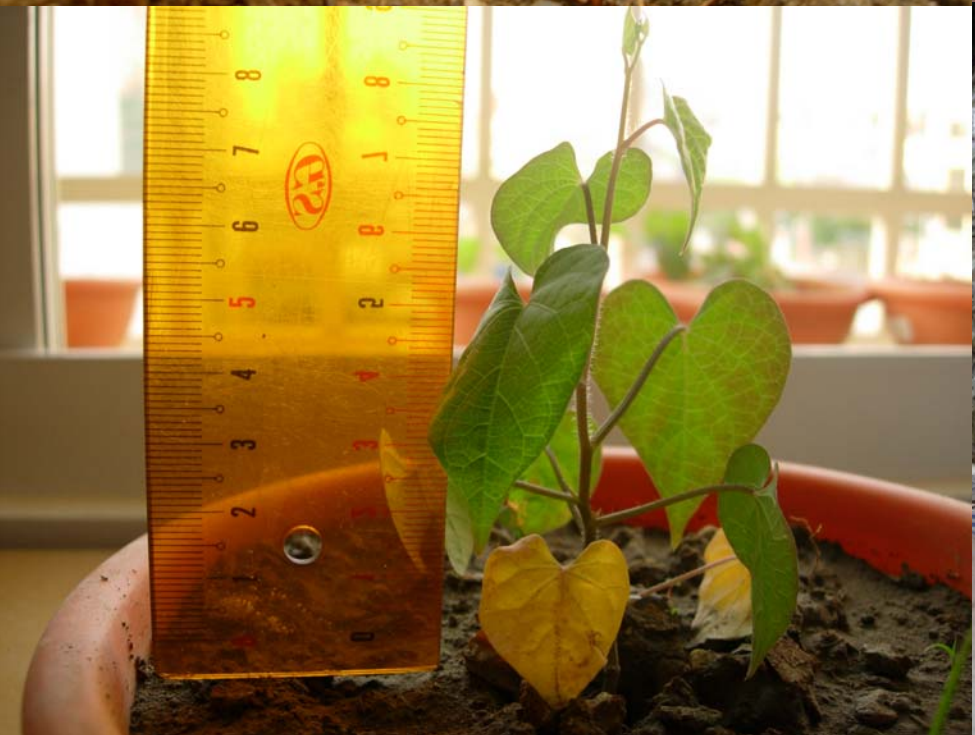
AMF Tests (2/3)

- The soil used for this part of study was collected from the heavy metals contaminated area with electroplating industry, located in Hunei, Kaohsiung County, Taiwan.
- The areas were rich in various heavy metals, and the three elements with highest concentrations included Pb (3032 mg/Kg), Cu (232 mg/Kg) and Zn (1012 mg/Kg), which would be tested in this study.
- The tests were run in a greenhouse by using a 2×2×3 factorial completely randomised

AMF Tests (3/3)

- The two plant species used in this study were manipulated with and without inoculation by AM fungi (*G. macrocarpum*) in the contaminated sites.
- Eight weeks later, the plants in the pots were harvested for analysis.
- A field experiment was also conducted in the contaminated areas mentioned previously in this part of study in order to compare with the pot test.
- The experiment was operated under natural conditions in a 2×2×8 factorial completely randomised design, with eight replicates.
- The area of each field plot was 80cm×40cm, and the distance between two neighboring plots was 20 cm.


大花
大花



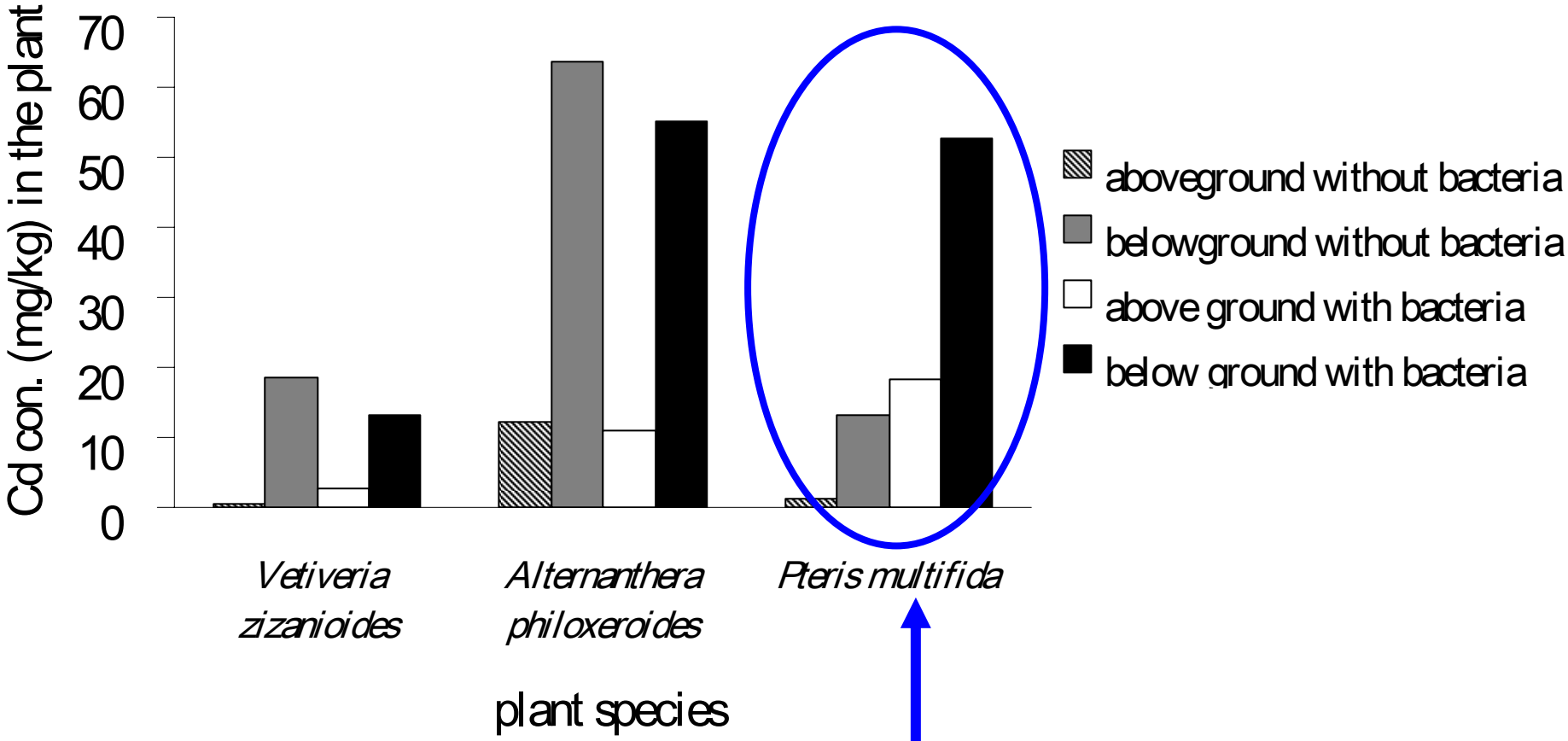



Statistical Analysis

- The results of the experiments were analysed by using two-way analysis of variance (ANOVA). Comparisons between the means were carried out by using the LSD (least significant difference) tests at a significance level of $P < 0.05$.

Results

PSB Tests



Con. of Cd in plants harvested at the 8th week, with 10 mg/kg of Cd contaminated soil.

***Pteris multifida* was significantly increased after PSB was added, especially in the aboveground parts**

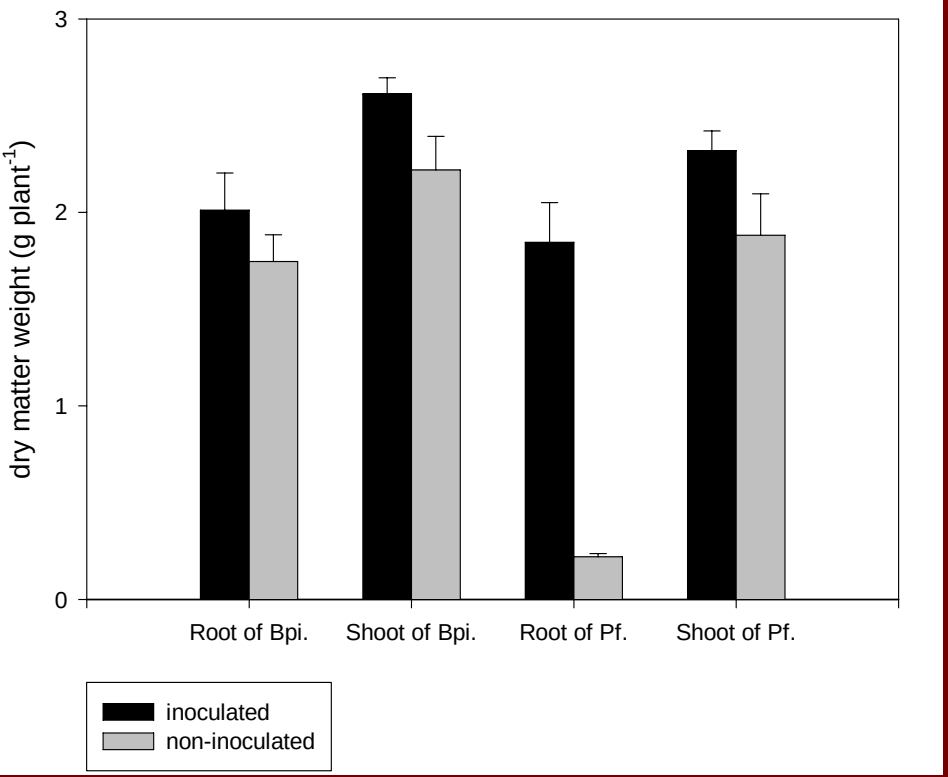
- However, *Pteris multifida* was still died finally.
- The reason might be that the Cd conc. in *Pteris multifida* exceeded its threshold concentration to survive.
- However, according to the study by Xiao *et al*. (2008), *Pteris vittata* can tolerate higher Cd conc. (> 80 mg/Kg).
- The function of PSB to promote growth of plants seem to be not suitable for every species.

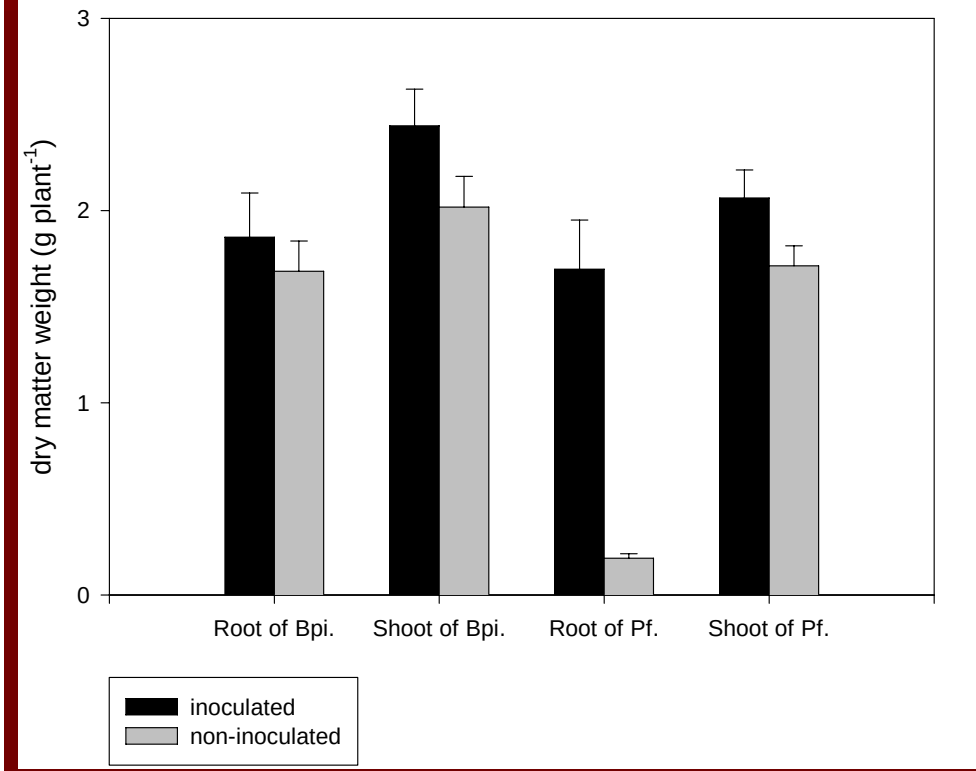
AMF Tests

Mycorrhizal colonisation Ratio

	Passiflora foetida	*Bidens pilosa*
Pot	71 (±2) %	68 (±3) %
Field	63 (±2) %	55 (±3) %

Effects of Inoculation on Plant Growth





Bidens pilosa **and** ***Passiflora foetida*** **were pronouncedly increased their biomass by the inoculation with AMF**

Statistical Analytical Results for AMF Tests

The effect of AMF on heavy metal con. of *Passiflora foetida*

Heavy metal	Inoculation	Experiment	Con. in shoot (mg kg^{-1})	Con. in root (mg kg^{-1})	Shoot/root ratio
	Inoculation	Pot	24.45 (±4.01)a	85.62 (±4.21)a	0.29
		Field	15.11 (±1.03)b	69.34 (±3.59)b	0.22
	Non-inoculated	Pot	22.37 (±3.38)a	53.61 (±7.44)c	0.42
Cu		Field	10.89 (±1.83)b	44.04 (±2.09)d	0.25
	F-level/significance				
Cu	Inoculation(A)		3.7 (n.s.)	109.2***	
	Experiment(B)		40.6***	22.2**	
	(A)× (B)		0.4 (n.s.)	1.5 (n.s.)	
	Inoculation	Pot	67.97 (±6.27)ab	1110.29 (±55.37)a	0.06
		Field	57.8 (±4.79)a	877.65 (±31.91)b	0.07
	Non-inoculated	Pot	72.38 (±8.54)b	632.94 (±104.21)c	0.11
Pb		Field	9.64 (±0.64)c	556.29 (±54.99)c	0.02
	F-level/.significance				
Pb	Inoculation(A)		42.3***	106.5***	
	Experiment(B)		117.5***	16.0**	
	(A)× (B)		61.1***	4.1 (n.s.)	
	Inoculation	Pot	78.09 (±2.46)a	179.85 (±20.42)a	0.43
		Field	63.17 (±4.32)b	157.63 (±7.83)b	0.40
	Non-inoculated	Pot	71.41 (±6.34)abc	158.85 (±3.65)ab	0.45
Zn		Field	59.3 (±11.16)bc	149.03 (±6.19)bc	0.40
	F-level/.significance				
Zn	Inoculation(A)		1.8 (n.s.)	5.0*	
	Experiment(B)		11.6**	5.8*	
	(A)× (B)		0.1 (n.s.)	0.9 (n.s.)	

S

R

- (P)
- (F)
+ (P)
+ (F)
- (P)
+ (F)
+ (P)
+ (F)
- (P)
- (F)

P: pot

F:field

The effect of AMF on heavy metal con. of *Bidens pilosa*

Heavy metal	Inoculation	Experiment	Con. in shoot (mg kg^{-1})	S	Con. in root (mg kg^{-1})	R	Shoot/root ratio
Cu	Inoculation	Pot	20.27 (±1.40)a		75.39 (±7.62) a		0.27
		Field	17.63 (±0.53)b		62.65 (±2.13) b		0.28
	Non-inoculated	Pot	17.56 (±0.55)b		63.57 (±7.98) b		0.28
		Field	10.53 (±1.04)c		50.38 (±0.8) c		0.21
	F-level/significance						
	Inoculation(A)		79.8***	+ (P)	13.7**	+ (P)	
	Experiment(B)		77.7***	+ (F)	15.9**	+ (F)	
	(A)× (B)		15.9**		0.1(n.s.)		
Pb	Inoculation	Pot	82.62 (±11.49)a		1127.72 (±230.74)a		0.07
		Field	23.34 (±7.42)b		896.95 (±59.57)b		0.03
	Non-inoculated	Pot	98.12 (±8.83)a		865.82 (±20.44)b		0.11
		Field	17.87 (±4.89)b		773.95 (±26.33)b		0.02
	F-level/.significance						
	Inoculation(A)		1.0 (n.s)	- (P)	7.7*	+ (P)	
	Experiment(B)		202.1***		5.4*		
	(A)× (B)		4.6 (n.s.)	- (F)	1.0 (n.s.)	+ (F)	
Zn	Inoculation	Pot	136.36 (±5.08)a		160.36 (±23.44)a		0.85
		Field	117.99 (±3.18)b		133.97 (±3.23) ab		0.88
	Non-inoculated	Pot	130.44 (±3.59)a		132.40 (±18.72)ab		0.99
		Field	112.44 (±8.58)b		116.92 (±4.53)b		0.96
	F-level/.significance						
	Inoculation(A)		3.2 (n.s.)	- (P)	6.5*	- (P)	
	Experiment(B)		32.4***	- (F)	5.7*	- (F)	
	(A)× (B)		0.1 (n.s.)		0.4 (n.s.)		

Cu

Pb

Zn

P: pot

F:field

Conclusion

Conclusions (1/2)

- Vetiveria was the best accumulator among the three plant species (*Vetiveria zizanioides*, *Pteris multifida*, and *Alternanthera philoxeroides*) for Cd
- The Cd con.in *Pteris multifida* was found significantly increased after adding PSB, but exceeded threshold concentration
- PSB seemed to be not suitable for every plant species.

Conclusions (2/2)

- ***Bidens pilosa*** inoculated with AMF had significantly higher conc. of Cu and Pb than non-inoculated plants in the both shoots and roots.
- ***Passiflora foetida*** inoculated with AMF also had significantly higher Cu and Pb, but only in the root.
- Both of ***Bidens pilosa*** **and** ***Passiflora foetida*** inoculated with AMF increased dramatically for total amounts of Cu, Pb and Zn comparing to the non-inoculated ones.
- Translocation proportion from root to shoot was Zn>Cu>Pb.
- Pot experiment were higher in comparison to the field experiment

Thank You for
Your Attention.