MICROBIOLOGY INDONESIA
ISSN 1978-3477, eISSN 2087-8575
Vol 7, No 4, November 2013, p 167-176

Available online at
http://jurnal.permi.or.id/index.php/mionline
DOI: 10.5454/mi.7.4.5

The Role of Arbuscular Mycorrhizal Fungus (*Gigaspora margarita*) on Mercury and Nutrients Accumulation by *Enterolobium cyclocarpum* Seedlings

HANNA ARTUTI EKAMAWANTI[1,2], YADI SETIADI[3]*, DIDY SOPANDIE[4], AND DWI ANDREAS SANTOSA[5]

[1]*Tropical Silviculture Major, Post-graduate School, Institut Pertanian Bogor, Jalan Lingkar Akademik Kampus IPB Darmaga, Bogor 16680, Indonesia;*
[2]*Faculty of Forestry, Universitas Tanjungpura, Jalan Imam Bonjol, Pontianak, Indonesia;*
[3]*Laboratory of Forest Biotechnology, Research Center for Biological Resources and Biotechnology, Institut Pertanian Bogor, Jalan Lingkar Akademik Kampus IPB Darmaga, Bogor 16880, Indonesia;*
[4]*Department of Agronomy and Horticulture, Institut Pertanian Bogor, Jalan Meranti, Kampus IPB Darmaga, Bogor 16680, Indonesia;*
[5]*Laboratory of Biotechnology Soil and Environment, Department of Soil and Land Resources, Institut Pertanian Bogor, Jalan Meranti, Kampus IPB Darmaga, Bogor 16680, Indonesia*

A river-sand culture experiment was conducted to investigate whether arbuscular mycorrhizal (AM) colonization influenced mercury (Hg) and nutrients accumulation, and whether AM fungus (AMF) *Gigaspora margarita* enhance host plant sengon buto (*Enterolobium cyclocarpum*) tolerance to Hg. Hg was applied as $HgCl_2$ at different levels (375 and 750 µM) and added to the full strength of Hoagland's solution, then applied to seedlings in river-sands as growth media according to treatments. The non-mycorrhizal and mycorhizal *E. cyclocarpum* roots took up Hg, but its translocation to the leaves was inhibited. AM inoculation decreased significantly Hg content of roots seedlings by 70.5% from non-AM inoculation seedlings. Mycorrhizae enhanced significantly Ca and Mg uptake in shoot by 1.29- and 1.27-fold higher than non-mycorrhizal seedlings, but not enhanced significantly P uptake. Based on the roots dry weight, the tolerance index of non-mycorrhizal or mycorrhizal seedlings treated with 750 µM Hg supply was > 50%. It indicated that the seedlings can tolerate up to 750 µM Hg added. Considering the possible differences in AMF response to Hg in polluted soil from the field, it is not yet clear if *Gi. margarita* could be applied for phytoremediation of Hg in contaminated sites. Therefore, more work needs to be done using AMF isolates to reveal the possible application in the management of Hg contaminated soils.

Key words: arbuscular mycorrhizal fungus, *Enterolobium cyclocarpum, Gigaspora margarita,* mercury

Percobaan untuk meneliti apakah kolonisasi mikoriza arbuskula (MA) mempengaruhi akumulasi merkuri (Hg) dan unsur hara, dan apakah fungi MA (FMA) *Gigaspora margarita* meningkatkan toleransi tanaman inang Sengon Buto (*Enterolobium cyclocarpum*) terhadap Hg telah dilakukan. Perlakuan Hg diberikan dengan konsentrasi berbeda (375 dan 750 µM $HgCl_2$) yang ditambahkan ke dalam larutan hara Hoagland *full strength*, kemudian diberikan pada semai di media tumbuh pasir sungai sesuai perlakuan. Akar *E. cyclocarpum* tidak bermikoriza dan bermikoriza menyerap Hg, tetapi translokasinya ke daun dihambat. Inokulasi MA nyata menurunkan kadar Hg akar semai 75% dari semai yang tidak diinokulasi MA. Mikoriza nyata meningkatkan serapana Ca dan Mg di tajuk 1.29 dan 1.27 kali lebih tinggi dari semai tidak bermikoriza, tetapi tidak nyata meningkatkan serapan P. Berdasarkan bobot kering akar, indeks toleransi semai tidak bermikoriza dan bermikoriza yang diberi perlakuan Hg 750 µM lebih dari 50%. Hal ini mengindikasikan bahwa semai dapat toleran Hg hingga 750 µM. Pertimbangan pada kemungkinan perbedaan respons FMA terhadap Hg di tanah tercemari dari lapangan, masih belum jelas apakah *Gi. margarita* dapat diaplikasikan untuk fitoremediasi lahan terkontaminasi Hg. Oleh karena itu, penelitian lebih lanjut dengan memanfaatkan isolat-isolat FMA diperlukan untuk mengembangkan kemungkinan aplikasinya dalam pengelolaan tanah-tanah terkontaminasi Hg.

Kata kunci: *Enterolobium cyclocarpum*, fungi mikoriza arbuskula, *Gigaspora margarita,* merkuri

Numerous soils have been polluted by mercury (Hg) as a result of anthropogenic activities, such as amalgamation of gold in gold-mining and the agricultural soils. Hg is a toxic chemical which is not degraded because it is an element (US EPA 2009). Management of the areas, which have been exposed to either intense or diffuse Hg pollution, has therefore become a major environmental concern. Strategies oriented toward the use of plants and microbes, or both in combinations within the plant rhizosphere, have been proposed in the recent years as an effective cleanup technology in removing or stabilizing heavy metals in polluted soils.

Among these microbes, arbuscular mycorrhizal

*Corresponding author; Phone/Fax: +62-251-8626178, Email: hanna.artuti@gmail.com

fungi (AMF) are particular interest due to their unique position at the soil/root interface and their recognized role in element transport and immobilization (Smith and Read 2008). AMF can tolerate a wide range of metal concentrations in soils (González-Guerrero *et al*. 2008), affect the accumulation of metals such as Cu, Cd, Zn, and As by plants and enhance the tolerance of host plants to these metals exposure in soil (González-Chávez *et al*. 2004; Janoušková *et al*. 2006; Marques *et al*. 2006). However, few studies have addressed the interaction between AMF and Hg in growth medium- or soil-plant system. Therefore, the potential of AMF has not yet been fully explored with respect to its Hg phytoremediation. The first report about the effects of AM inoculation on Hg behavior in soil-plant system is done by Yu *et al*. (2010) and it has been shown that Hg uptake was lower by mycorrhizal roots of maize than by non-mycorrhizal roots. Hg accumulation in several plants has been studied, such as white clover (Greger et al. 2005), alfalfa (Ortega-Villasante *et al*. 2005), lentil, chickpea (Rodríguez *et al*. 2007) and common vetch (Sierra *et al*. 2008). Nevertheless, very few studies have been conducted on the Hg tolerance in tropical trees, such as Sengon Buto (*Enterolobium cyclocarpum).* This species is one of the largest trees in the dry forest formation and a nitrogen-fixing species (World Agroforestry Centre 2013), therefore it could be a soil improver. If this tree species is proposed for phytoremediation on Hg-polluted soil, plant Hg uptake and resistance response should be evaluated under controlled conditions, prior to field establishment a screen to identify suitable candidate species.

The interaction between AMF, tree species (such as *E. cyclocarpum*) and Hg was the subject of this study because of the possibility of the beneficial effect of mycorrhizae in improving the resistance of plants against Hg toxicity. The study was carried out to determine the Hg and nutrient accumulation in *E. cyclocarpum* seedlings inoculated with AMF *Gigaspora margarita* (collection of Laboratory of Forest Biotechnology, Research Center for Biological Resources and Biotechnology, Bogor Agricultural University) grown in artificial Hg-polluted river sands media in pot experiment. Such knowledge would help clarify the potential of tree species *E. cyclocarpum* and AMF as phytoremediation agents of Hg-polluted soils.

MATERIALS AND METHODS

Plant Culture in River Sands at Different Rates of Hg. *E. cyclocarpum* seeds were surface-sterilized by immersing the seeds in 0.05% NaOCl for 60 sec and then rinsing them three times with distilled water (dH_2O). To break dormancy, the seeds were soaked in boiling water for 5 min and then in dH_2O overnight. The seeds were germinated on zeolite media. After formation of completed leaves, a seedling was transplanted on to zeolite media in polybag (15 cm x 25 cm). Mycorrhizal seedlings were obtained by inoculating the seedlings with *Gi. margarita* inoculums (Laboratory of Forest Biotechnology, Research Center for Biological Resources and Biotechnology, Bogor Agricultural University collection) as first inoculation First inoculation of AMF inoculum when they were germinated and transplanted on zeolite. Each nine-months old seedlings of non-mycorrhizal and mycorrhizal was grown in a PVC pot (10 cm in diameter and 30 cm in height), containing 5 kg coarse river sands (1-2 mm) as substrate and maintained in screen house. *Gi. margarita* inoculum (25 g) was inoculated to mycorhizal seedlings for second inoculation and sterilized mycorrhizal inoculum (25 g) and its filtrate (10 mL each pot) was applied to non-mycorrhizal seedlings. All seedlings were maintained by adding the nutrient solution for 8 weeks. The composition of the nutrient solution was as follows (mmol L^{-1}) (Zornoza et al. 2010): $Ca(NO_3)_2$ 1.5; KNO_3 4.0; KH_2PO_4 1.5 and $MgSO_4$ 1.0. Micronutrients were supplied (µmol L^{-1}): Fe-EDDHA 36; $MnSO_4.H_2O$ 33; $ZnSO_4.7H_2O$ 1.6; $CuSO_4.5H_2O$ 1.6; H_3BO_3 46, and $(NH_4)_6Mo_7O_{24}.4H_2O$ 0.1. The pH of nutrient solution ranged for 5.5 to 6.0. A factorial experiment with completely randomized design with three replicates for each treatment was used. Hg treatments were 0, 375 and 750 µM, supplied as $HgCl_2$ salt and applied to *E. cyclocarpum* seedlings, either with or without AM inoculation treatments. These concentration of Hg treatments were threshold concentration of Hg toxicity for *E. cyclocarpum* seedlings based on the result of previous research. The experiment was carried out in screen house of Forest Microbiology Laboratory, Agency for the Research of Forest Rehabilitation and Conservation, Bogor, under the following conditions: 28 °C-33 °C temperature and 80%-95% humidity.

Harvesting. Plants were harvested after 30 d to Hg exposure and divided into roots, stems and leaves. All plant material was thoroughly washed with tap water followed by a subsequent rinse in 20 mM EDTA solution for 1 min and then in deionized water for 2 min. Total fresh weight of each tissue was determined, then a representative plants were oven-dried to

constant weight at 70 °C for 3 d prior to preparation for measuring dry weights and nutrients analysis, and at 50 °C prior to preparation for Hg analysis. Water content was determined based on the difference of fresh weight and dry weight. The rest of the material plants was frozen in liquid N_2 and stored at -20 °C before malondialdehyde (MDA) concentration analyzing.

Analytical determination. Dried plant sample was acid digested using 25 mL of deionized water, 3.5 mL of concentrated HNO_3 and 0.5 mL of $HClO_4$, added to 0.5 g dry weight (DW) in a test tube. The digested solution was analyzed for Hg concentration using inductively coupled plasma-optical emission spectrometry (ICP-OES, Spectro Genesis) excitation, and calibration was performed by using standard Hg solution (Han *et al*. 2006).Total Hg and nutrient (P, Ca, and Mg) concentration were determined by ICP-OES. Lipid peroxidation in plant tissues was based on an estimate of malondialdehyde (MDA) concentration, as described by Heath and Packer (Ortega-Villasante *et al*. 2005). AM colonization was determined following Brundrett *et al*. (1996).

Calculation. Some variables were calculated to study the resistance to mercury stress of *E. cyclocarpum* seedlings (Moreno-Jiménez *et al*. 2007):

$$\text{Hg content in roots (ng roots}^{-1}\text{) and leaves (ng leaves}^{-1}\text{)} = [\text{Hg}] \times \text{g DW}$$

where [Hg] was Hg concentration, and DW was the roots or leaves dry weight.

$$\text{Nutrient uptake (nmol g}^{-1}\text{ DW)} = \frac{\text{nmol plant}^{-1}}{\text{g DW}}$$

Nutrients (P, Ca and Mg) uptake and translocation were calculated as (Wang and Greger 2004):

$$\text{Nutrient translocation (nmol Hg g}^{-1}\text{ DW)} = \frac{\text{nmol leaves}^{-1}}{\text{g DW}}$$

where DW was the entire-plant dry weight.

Tolerance index (TI) of mycorrhizal and non-mycorrhizal seedlings to Hg treatments was determined based on roots dry weight, as (Rabie 2005):

$$\text{TI (\%)} = \frac{\text{DW of roots at Hg treated}}{\text{DW roots at non Hg treated of the same treatments}} \times 100$$

Water content (WC) of leaves, stems or roots was calculated as:

$$\text{WC (\%)} = \frac{\text{(FW-DW) of leaves, stems, or roots}}{\text{FW of leaves, stems, or roots}} \times 100$$

where FW was fresh weight.

Statistical analyzes. A statistical analyzes for means comparison was carried out using ANOVA and least significant difference (LSD) test using statistical software CoStat 6.400.

RESULTS

Mercury Accumulation. Hg accumulation in *E. cyclocarpum* seedlings was affected significantly by Hg treatment. Hg concentration in roots of *E. cyclocarpum* seedlings was 8- to 16-fold and Hg content in roots was 3- to 6-fold higher than the control (without Hg) (Table 1). However, at the 750 µM of Hg, Hg accumulation in roots was decreased significantly by 49.6% of Hg concentration and 57.2% of Hg content when compared to Hg accumulation in roots of seedlings treated with 375 µM of Hg. Hg accumulation in roots without Hg treatment shows that there was Hg cross contamination in the air. Table 1 shows that almost there are not Hg accumulation in leaves of *E. cyclocarpum*. On the other hand, AM inoculation decreased Hg content of roots seedlings by 70.5% from non-AM inoculation seedlings (data not shown).

Nutrients Accumulation and Distribution. The highest Hg concentration had stronger effects regarding nutrients translocation than regarding nutrients uptake, especially in mycorrhizal seedlings (Table 2). The supply of 750 µM Hg caused reducing in P and Mg translocation by 53% and 47%, respectively, lower than the control (without Hg supply) in mycorrhizal seedlings. P uptake also followed a similar response to highest Hg rate by 29% lower than the control in seedlings inoculated with AMF. In contrast, nutrients (P, Ca, and Mg) uptake and translocation were not difference in non-mycorrhizal seedlings treated with the Hg supply. Moreover, nutrients uptake and translocation did not suffer decreases in seedlings at the lower Hg supply (375 µM), either in non-mycorrhizal or mycorrhizal seedlings.

Table 2 also showed that AM inoculation had negative effects regarding nutrients translocation, but not regarding nutrients uptake in seedlings treated with the Hg supply. The AM inoculation caused decreases significantly in P and Mg translocation to leaves by 56% and 44%, respectively, lower than in non-mycorrhizal seedlings treated with 750 µM Hg apply. In contrast, P and Mg uptake showed no difference effects of between control (no AM inoculation) and AM inoculation in seedlings at 375 µM Hg supply. This

Table 1 Hg concentration and Hg content in roots and leaves of *E. cyclocarpum* seedlings grown for 30 d in river sands with treatment of different Hg concentration

Hg (μM)	Hg concentration (ng g^{-1} DW)		Hg content (ng)	
	Roots	Leaves	Roots	Leaves
0	38.2 ± 26.2 a	1.8 ± 3.0	363.5 ± 467.4 a	9.6 ± 14.7
375	611.0 ± 124.3 c	n.d	2,066.5 ± 1,759.0 c	n.d
750	302.8 ±154.8 b	n.d	1,183.0 ± 991.1 b	n.d

Significant differences among Hg treatments are indicated by different letter (mean ± SD, n = 6; LSD's test, $p < 0.05$). DW: dry weight.

Table 2 Nutrients uptake and translocation in *E. cyclocarpum* seedlings grown for 30 d in river sands with treatments of different Hg concentrations and AM inoculation

Hg (μM)	AM inoculation			
	Without	With	With out	With
	------ P uptake (μmol g^{-1} DW) -------		------- P translocation (μmol g-1 DW) --------	
0	109.8 ± 23.3 a(B)	164.7 ± 14.8 a(A)	19.3 ± 7.3 a(A)	29.2 ± 8.3 a(A)
375	127.0 ± 8.1 a(A)	162.5 ± 21.5 a(A)	23.4 ± 6.0 a(A)	28.5 ± 16.1 a(A)
750	142.1 ±36.7 a(A)	116.2 ±21.2 b(A)	30.2 ± 0.7 a(A)	13.3 ± 2.5 b(B)
	-------- Ca uptake (μmol g^{-1} DW) --------		-------- Ca translocation (μmol g^{-1} DW) -------	
0	60.0 ± 3.3 a(A)	84.3 ± 14.3 a(A)	65.1 ± 21.8 a(A)	93.4 ± 30.5 a(A)
375	47.7 ± 2.6 a(A)	60.9 ± 12.2 a(A)	54.1 ± 10.3 a(A)	61.7 ± 18.9 a(A)
750	50.0 ± 3.2 a(A)	58.7 ± 3.6 a(A)	68.6 ±15.0 a(A)	42.6 ± 11.6 a(A)
	-------- Mg uptake (μmol g^{-1} DW) --------		------- Mg translocation (μmol g^{-1} DW) -------	
0	23.3 ± 2.3 a(A)	34.5 ± 8.6 a(A)	67.9 ± 19.7 a(A)	102.4 ± 32.1 a(A)
375	24.4 ± 1.3 a(A)	31.5 ± 2.2 a(A)	75.5 ± 15.5 a(A)	88.6 ± 31.1 a(A)
750	23.3 ±6.4 a(A)	24.6 ± 7.3 a(A)	83.6 ± 3.8 a(A)	46.9 ± 8.3 b(B)

Significant differences among Hg treatments are indicated by small different letter and among AM inoculation treatments with different capital letters (mean ± SD, n = 3; LSD's test, $p < 0.05$). DW: dry weight.

tendency was also found regarding Ca uptake and translocation. On the other hand, AM inoculation increased P uptake, significantly, and tended to increase Ca and Mg uptake and also nutrients translocation in seedlings treated without Hg supply. In addition, AM inoculation as a single factor treatment also enhanced Ca and Mg uptake in seedlings, significantly, by 1.29- and 1.27-fold higher than non-mycorrhizal seedlings, respectively (Table 3).

***E. cyclocarpum* Seedlings Biomass and MDA Concentration.** The negative effect of Hg in plants was reflected in biomass (dry weight), being significantly ($p < 0.05$) reduced in non-mycorrhizal and mycorrhizal seedlings, when cultivated with Hg supply (Table 4). Dry weight of leaves, stems and roots decreased in the range 38-54%, 54-67%, and 43-54%, respectively. It indicated that there was reduction of plant growth caused by Hg supply up to 750 μM during 30 d of Hg exposure.

On the other hand, biomass of seedling treated with AM inoculation was decreased significantly (Table 5). In stems and roots, *E. cyclocarpum* seedlings treated with AM inoculation showed 63% and 68% decrease in the biomass respectively, but a sharp decrease (72%) was occurred in leaves.

Regarding water content (Table 6), the effect of AM inoculation depended on Hg treatment. At control (without Hg supply), leaves and stems water content in

Table 3 Nutrients uptake in *E. cyclocarpum* seedlings grown for 30 d in river sands with treatment of mycorrhizal

AM inoculation	P uptake (μmol g^{-1} DW)	Ca uptake (μmol g^{-1} DW)	Mg uptake (μmol g^{-1} DW)
Without	126.3 ± 26.2 a	52.6 ± 6.3 b	23.7 ± 3.5 b
With	147.8 ± 29.1 a	68.0 ± 15.6 a	30.2 ± 7.2 a

Significant differences among Hg treatments are indicated by different letter (mean ± SD, n = 9; LSD's test, $p < 0.05$). DW: dry weight.

Table 4 Leaves, stems and roots dry weight of *E. cyclocarpum* seedlings grown for 30 d in river sands with treatments of different Hg concentrations

Hg (μM)	Dry weight (g)		
	Roots	Stems	Leaves
0	7.0 ± 4.8 a	11.1 ± 5.6 a	3.7 ± 2.2 a
375	3.2 ± 2.5 b	3.7 ± 2.7 b	1.7 ± 1.6 b
750	4.0 ± 2.9 b	5.1 ± 4.4 b	2.3 ± 2.1 b

Significant differences among Hg treatments are indicated by different letter (mean ± SD, n = 6; LSD's test, $p < 0.05$). DW: dry weight.

mycorrhizal seedlings were increased by 11% each. Even though AM inoculation did enhance leaves water content, it increased stems water content significantly by 27% when the seedlings were exposed to 750 μM Hg. Moreover, mycorrhizal inoculation enhanced roots water content, 17% higher than non-mycorrhizal, in seedlings treated with or without Hg treatments (data not shown).

Hg exposure for 30 d increased MDA concentration in roots of *E. cyclocarpum* seedlings, significantly (Fig 1). MDA concentration of roots increased when the seedlings were grown in Hg-treated river-sand media, either with or without AM inoculation. AM inoculation caused the highest increase in MDA concentration by 485% in the roots of seedlings treated with 750 μM Hg, when compared to the control (without AM inoculation). On the other hand, 375 μM and 750 μM Hg supply resulted in the increased MDA concentration in leaves by 14% and 97%, respectively (data not shown).

Tolerance index of *E. cyclocarpum* seedlings grown for 30 d in river sands treated with 375 μM Hg was < 50% but the tolerance index was > 54% when the seedlings were treated with 750 μM (Table 7). Roots of non-inoculated seedlings remained non-mycorrhizal while roots of inoculated ones were colonized by AMF (Table 7). The low AMF colonization in seedlings treated with Hg indicated that *Gi. margarita* colonization was reduced in the presence of Hg in substrate.

DISCUSSION

The results obtained during this research have shown that AMF *Gi. margarita* decreased Hg content in root seedlings. We predicted that decreasing Hg content in roots is one of Hg tolerance mechanisms in mycorrhizal symbiosis. This result was in line with Yu *et al*. (2010), AMF *Glomus mosseae* decreased significantly Hg concentration in maize roots when Hg was applied at the rates of 2.0 dan 4.0 mg kg^{-1}. All interactions of Hg with soil/growth media, roots, and AMF influence its uptake by plants, but this presume need to be investigated in further research. On the other hand, Hg accumulation in roots treated with 750 μM of Hg supply decreased significantly by 49.6% of Hg concentration and 57.2% of Hg content when compared to Hg accumulation in roots of seedlings treated with 375 μM of Hg. It shows that at the highest Hg treatment, Hg accumulation in root seedlings was limited. This result was in contrast with Esteban et al. (2008) result, which found the pattern of long-term (over 28 d) Hg accumulation in shoots and roots white lupin (*Lupinus albus* L.) can be dissected into linear and hyperbolic (saturable) components, when they were grown in 5 and 10 μM Hg supply in hydrophonics. The result of experiment also showed that a main part of Hg accumulated by *E. cyclocarpum*

Table 5 Leaves, stems and roots dry weight of *E. cyclocarpum* seedlings grown for 30 d in river sands with treatments of AM inoculation

AM inoculation	Dry weight (g)		
	Roots	Stems	Leaves
Without	7.2 ± 3.7 a	9.6 ± 5.1 a	3. 9 ± 1.9 a
With	2.3 ± 1.4 b	3.6 ± 3.6b	1.1 ± 0.9 b

Significant differences among Hg treatments are indicated by different letter (mean ± SD, n = 9; LSD's test, $p < 0.05$)

Table 6 Roots, stems, and leaves water content of *E. cyclocarpum* seedlings grown for 30 d in river sands with treatments of different Hg concentrations and AM inoculation

Hg (μM)	AM inoculation					
	Without	With	Without	With	Without	With
	Water content (%)					
	----------Roots ----------		---------- Stems ----------		---------- Leaves ----------	
0	56.1 ± 9.3 a(A)	73.3 ± 3.3 a(A)	61.3 ± 2.8 b(B)	68.0 a ± 2.1 a(A)	68.3 ± 2.9 b(B)	75.8 ± 0.3 a(A)
375	66.3 ± 4.0 a(A)	74.9 ± 7.2 a(A)	67.1 ± 2.8 a(A)	66.7 a ± 2.6 a(A)	73.3 ± 1.2 a(A)	72.3 ± 4.0 a(A)
750	69.2 ± 9.8 a(A)	76.3 ± 3.3 a(A)	61.1 ± 1.7 b(B)	77.6 a ± 9.9 a(A)	69.5 ± 0.5 ab(A)	68.5 ± 2.3 b(A)

Significant differences among Hg treatments are indicated by small different letter and among AM inoculation treatments with different capital letters (mean ± SD, n = 3; LSD's test, $p < 0.05$)

seedlings was in the roots. There was no translocation of Hg to the aerial part (leaves) even though the Hg concentration in the growth media was higher. It indicates that *E. cyclocarpum* is not an Hg accumulator, but an Hg excluder. This result in line with some studies which have indicated that only a very small amount of Hg is translocated to plant shoot after root uptake and Hg in leaf mainly comes from the uptake of air Hg (Ericksen *et al.* 2004; Greger *et al*. 2005; Fay *et al.* 2007; Chen *et al*. 2009). Even though it was not an Hg accumulator, the potential of *E. cyclocarpum*as an Hg phytoremediation agent could be explored because the roots accumulated Hg. Plants that accumulate Hg in the roots convert the pollutant into less available forms, thus they could be used to prevent Hg leaching and moving to other place.

In contrast, arbuscular mycorrhizal symbiosis tend to enhance nutrients (P, Ca, and Mg) uptake and translocation, especially in seedlings treated without Hg supply and 375 μM Hg supply (Table 2). Nevertheless the enhancement was not significant but as a single factor, AM inoculation enhanced P, Ca and Mg uptake, significantly (Table 3). It is well documented that AM symbiosis can increase plant growth and nutrient uptake (Smith *et al*. 2008). However, AM symbiosis decreased nutrients (P and Mg) translocation in seedlings treated with the highest Hg supply (750 μM). We predict that disturbances of water fluxes altered P and Mg distribution due to high concentration Hg in growth substrate and also due to the existence of P and Mg competition between plant and AMF *Gi. margarita* in a limited space of pot experiment. Although a contribution of concentration effects after Hg supply cannot be ruled out, the increase of some nutrients could also be a strategy to avoid toxicity in plants (Moreno-Jiménez *et al*. 2007). In other case, the increase of nitrogen by nitrogen supply prevents oxidative stress in roots (Carrasco-Gil *et al*. 2012).

Hg can induce toxicity symptoms in plants such as inhibition of plant growth or disturbances on water and nutrient uptake (Patra *at al.* 2000). Mercury accumulated in plants evokes severe phytotoxicity and impairs numerous metabolic processes including nutrient uptake, water status, and photosynthesis (Chen *et al.* 2012). The inhibition of plant growth might be the first symptom of Hg stress (Cho *et al.* 2000). It was showed that Hg decreased significantly dry weight of

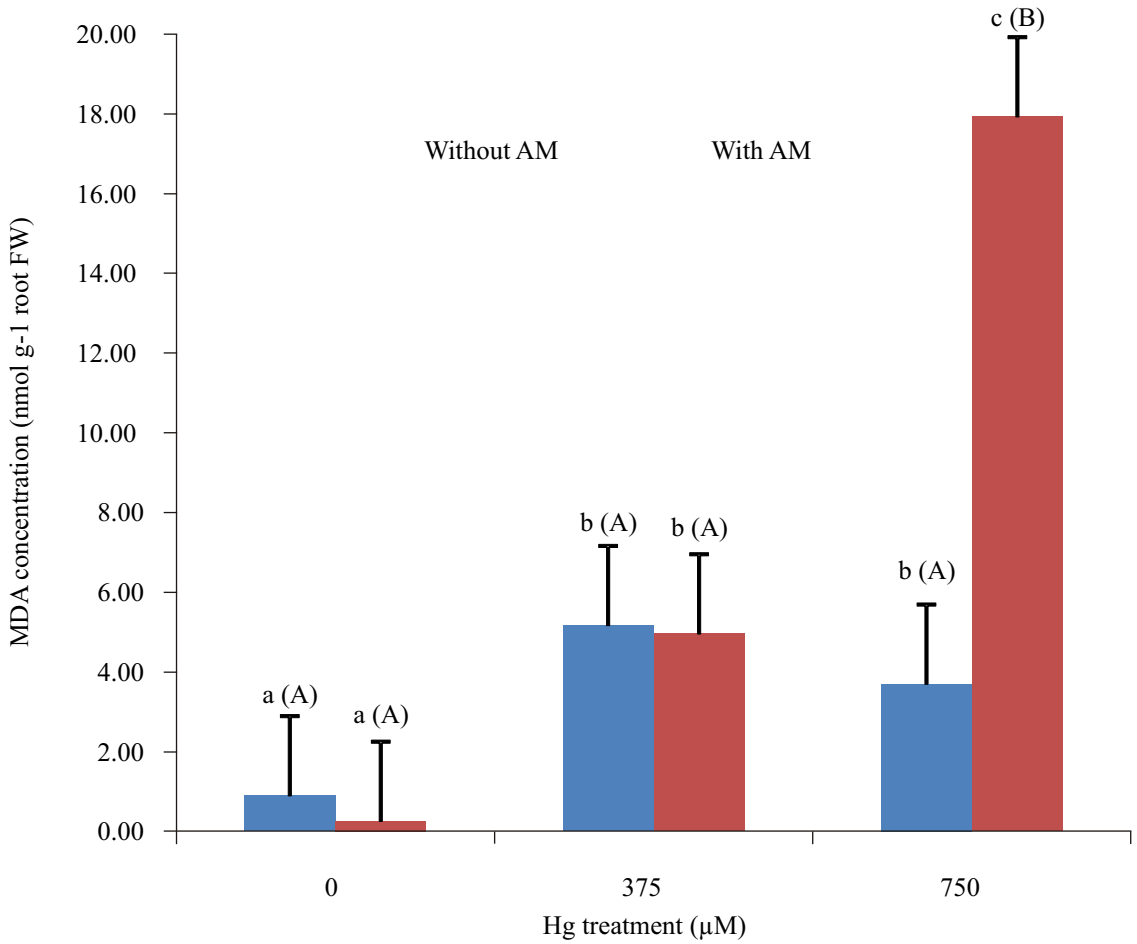

Fig 1 MDA concentration in roots of *E. cyclocarpum* seedlings with different Hg concentration (mean ± SD, n = 3). Significant differences among Hg treatments are indicated by small different letter and among AM inoculation treatments with different capital letters (LSD's test, $p<0.05$).

Table 7 Tolerance index and AM colonization of *E. cyclocarpum* seedlings grown for 30 d in river sands with treatments of different Hg concentrations and AM inoculation

Hg (µM)	AM inoculation			
	Without	With	Without	With
	---------- Tolerance index* (%) ----------		------ AM colonization (%) -----	
0	100	100	0	65.9
375	46.3	45.6	0	47.2
750	54.2	66.9	0	40.8

Remark: * The tolerance index was based on roots dry weight

roots, stems, and leaves of *E. cyclocarpum* seedlings during 30 d exposure to Hg supply (Table 4). The reduction of plant growth caused by Hg also been shown for *Rumex induratus* and *Marrubium vulgare* (Moreno-Jiménez *et al.* 2007). Generally, the formed mycorrhizal symbiosis significantly improved plant growth performance, such as plant height, stem diameter, shoot, root or total dry weight. In contrast, as a single factor of AM inoculation decreased dry weight of roots, stems and leaves significantly (Table 5), even though AM inoculation increased P, Ca, and Mg uptake (Table 3). We predicted that increase in P, Ca, and Mg uptake might be not followed by increase in other macro and micro nutrients. Therefore, increase in P, Ca and Mg uptake was not followed by increase in plant dry weight. Additionally, growth depressions can be explained with respect to carbon (C) demand and P supply by the AMF, where C drain to AMF was not

imbalance to P supply from AMF (Grace et al. 2009). Kiers et al. (2011) stated that plants can detect, discriminate, and reward the best fungal symbionts with more carbohydrates and in turn, their fungal symbionts enforce cooperation by increasing nutrient transfer only to those roots providing more carbohydrates.

It has been addressed that Hg can disturbed water fluxes, therefore, it can alter nutrients distribution which depends on water movement within the xylem (Moreno-Jiménez *et al.* 2007). Considering now water content, in each organ of *E. cyclocarpum* seedlings did not suffer reduction in water content during Hg exposure. It indicated that water movement within the seedlings was not disturbed by Hg apply. Therefore, nutrients distribution (P, Ca, and Mg) in *E. cyclocarpum* seedlings were not inhibited, especially when the seedlings exposed to Hg up to 375 µM concentration. This research only analyzed P, Ca, and Mg concentration, not other nutrients. Therefore, we cannot state if other nutrients translocation is affected. The role of AM inoculation to increase the water content of roots might be as an indirect strategy of seedlings to overcome Hg-induced water stress when the roots contacted Hg directly. It has been known, extraradical hyphae of AMF can also increase water uptake in addition to nutrients uptake (Smith *et al.* 2008).

MDA is one of organic compounds has been used as a biomarker, which can be useful in the early diagnosis of metal toxicity (Prasad 2003). In our research, AM inoculation enhanced MDA concentration in roots, significantly, higher than non-mycorrhizal seedlings when exposed to the highest Hg (750 µM) treatment. It suggested some degree of oxidative stress, probably due to the high Hg concentration in the roots without any increase of the antioxidant (Moreno-Jiménez *et al*. 2007) in roots of mycorrhizal seedlings. In contrast, MDA concentration in roots of mycorrhizal seedlings was not different with roots of non-mycorrhizal seedlings when exposed to the lower Hg (375 µM) supply. Increases of MDA concentration by Hg exposure have been observed in some previous results research (Cho *et al*. 2000; Ortega-Villasante *et al*. 2005; Moreno-Jiménez *et al*. 2006; Esteban *et al.* 2008). However, it has not clear yet how its mechanism, especially in mycorrhizal plants when they were exposed to Hg and there is no information available, therefore further investigation is still needed. In other case, inoculation with AMF caused reduction in MDA content in comparison to salinized plants, indicating lower oxidative damage in the colonized plants (Latef *et al.* 2011). In our research, there was no effect of AM inoculation on MDA concentration in leaves but only Hg supply as a single factor resulted in the increased of MDA concentration in leaves, nevertheless, there was no Hg accumulation in leaves. It was probably due to indirect effect of Hg accumulation in roots. This presumption has to be proved in further study.

Roots of inoculated plants were colonized by AMF, while non-inoculated controls remained non-mycorrhizal (Table 7). However, the percentage of AM colonization was reduced in the presence of Hg. This result indicated that the concentration of Hg supply in the substrate was harmful to AMF *Gi. margarita*. This finding is in line with Rabie (2005) who reported that sensitivity of AM symbionts to heavy metal polluted soil expressed as a reduction in root colonization. In contrast, addition of Hg in soil did not significantly influence maize root colonization rate (Yu *et al.* 2010). Another interesting result showed at Table 7, the presence of AMF could increase the metal tolerance index of *E. cyclocarpum* seedlings compared with non-mycorrhizal seedlings when they treated with 750 µM Hg. This result emphasizes that AMF could be potentially effective in protecting seedlings exposed to high levels of Hg concentration. The AMF ability to alleviate heavy metals stress of plants grown in heavy metal polluted soil was previously proved by Rufyikiri *et al*. (2000); Hildebrandt *et al*. (2007). In contrast, tolerance index of mycorrhizal or non-mycorrhizal seedlings grown for 30 d in river sands treated with 375 µM Hg was lower than seedlings treated with 750 µM (Table 7). It might be related to Hg accumulation in roots treated with 375 µM Hg was higher than in roots treated with 750 µM Hg (Table 1) then resulted in the decreased of roots dry weight.

In conclusion, this study provides further evidence for the protective effects of AMF *Gi. margarita* on *E. cyclocarpum* against Hg contamination, i.e. non significant effect on Hg accumulation in roots, a tendency to increase nutrient uptake and translocation when seedlings treated with Hg supply up to 375µM, the sharp decrease of dry biomass in seedlings, no different effect on water content in seedlings treated with Hg supply and no increase in MDA concentration in seedlings treated with 375 µM. It suggested that the role of symbiosis AMF *Gi. margarita* and *E. cyclocarpum* seedlings can tolerate up to 375 µM Hg supply. Even though, it is not yet clear if *Gi. margarita* could be applied for phytostabilization of Hg in contaminated sites. Considering the possible

differences in AMF response to Hg in polluted soil from the field, more work needs to be done with AMF isolates that originate from Hg-contaminated environments, to reveal the possible application of AMF in the management of Hg contaminated soils. Due to little information available on the potential role of AMF in plant accumulation of Hg and mechanisms involved, further studies are warranted.

REFERENCES

Brundrett M, Bougher N, Dell B, T Grove, Malajczuk N. 1996. Working with Mycorrhizas in Forestry and Agriculture. ACIAR Monograph 32.374 + x p.

Carrasco-Gil S. Estebaranz-Yubero M, Medel-Cuestab D, Millán R, Hernández LE. 2012. Influence of nitrate fertilization on Hg uptake and oxidative stress parameters in alfalfa plants cultivated in a Hg-polluted soil. Environmental and Experimental Botany 75:16-24. doi:10.1016/j.envexpbot.2011.08.013.

Chen J, Yang ZM. 2012. Mercury toxicity, molecular response and tolerance in higher plants. BioMetals 25(5):847-857. doi:10.1007/s10534-012-9560-8.

Chen J, Shiyab S, Han FX, Monts DL, Waggoner CA, Yang ZM, Su Y. 2009. Bioaccumulation and physiological effects of mercury in *Pteris vittata* and *Nephrolepsis exaltata.* Ecotoxocology 18(1):110-121. doi:10.1007/s10646-008-0264-3.

Cho UH, Park JO. 2000. Mercury-induced oxidative stress in tomato seedlings. Plant Sci. 156(1):1-9. doi:10.1016/S0168-9452(00)00227-2

Ericksen JA, Gustin MS. 2004. Foliar exchange of mercury as a function of soil and air mercury concentrations. Sci Total Environ. 324(1-3):271-279. doi:10.1016/j.scitotenv.2003.10.034.

Esteban E, Moreno E, Peñalosa J, Cabrero JI, Millán R, Zornoza P. 2008. Short and long-term uptake of Hg in white lupin plants: Kinetics and stress indicators. Environmental and Experimental Botany 62(3):316-322. doi:10.1016/j.envexpbot.2007.10.006.

Fay L, Gustin M. 2007. Assessing the influence of different atmospheric and soil mercury concentrations on foliar mercury concentrations in a controlled environment. Water Air Soil Poll. 181(1-4):373-384. doi:10.1007/s11270-006-9308-6.

González-Chávez MC, Wright SF, Nichols KA. 2004. The role of glomalin, a protein produced by arbuscular mycorrhizal fungi, in sequestering potentially toxic elements. Enviro Poll. 130(3):317-323. doi:10.1016/j.envpol.2004.01.004

González-Guerrero M, Melville LH, Ferrol N, Lott JNA, Azcón-Aguilar C, Peterson RL. 2008. Ultrastructural localization of heavy metals in the extraradical mycelium and spores of the arbuscular mycorrhizal fungus *Glomus intraradices*. Can J Microbiol. 54(2):103-110. doi:10.1139/W07-119.

Grace EJ, Smith FA, Smith SE. 2009. Deciphering the ArbuscularMycorrhizal Pathway of P Uptake in Non-responsive Plant Species. In:Azcón-Aguilar C, Barea JM, Gianinazzi S, Gianinazzi-Pearson V. Editors. Mycorrhizas-Functional Processes and Ecological Impact. Springer-Verlag Berlin Heidelberg, pp: 89-106. doi:10.1007/978-3-540-87978-7.

Greger M, Wang Y, Neuschútz C. 2005. Absence of Hg transpiration by shoot after Hg uptake by roots of six terrestrial plant species. Environ Poll. 134(2):201-208. doi:10.1016/j.envpol.2004.08.007

Han FXX, Banin A, Su Y, Monts DL, Plodinec MJ, Kingery WL, Triplett GE. 2002. Industrial age anthropogenic inputs of heavy metals into the pedosphere. Naturwissenschaften 89(11):497-504. doi:10.1007/s00114-002-0373-4.

Hildebrandt U, Regvar M, Bothe H. 2007. Arbuscular mycorrhiza and heavy metal tolerance. Phytochemistry 68(1):139-146. doi:10.1016/j.phytochem.2006.09.023.

Janoušková M, Pavlíková D, Vosátka M. 2006. Potential contribution of arbuscular mycorrhiza to cadmium immobilisation in soil. Chemosphere 65(11):1959-1965. doi:10.1016/j.chemosphere.2006.07.007.

Kiers ET, Duhamel M, Beesetty Y, Mensah JA, Franken O, Verbruggen E, Fellbaum CR,. Kowalchuk GA, Hart MM, Bago A, Palmer TM, West SA, Vandenkoornhuyse P, Jansa J, Bücking H. 2011. Reciprocal Rewards Stabilize Cooperation in the Mycorrhizal Symbiosis. Science 333(6044):880-882. www.sciencemag.org. doi:10.1126/science.1208473.

Latef AAHA, Chaoxing H. 2011. Effect of arbuscular mycorrhizal fungi on growth, mineral nutrition, antioxidant enzymes activity and fruit yield of tomato grown under salinity stress. Scientia Horticulturae 127(3):228-233. doi:10.1016/j.scienta.2010.09.020.

Marques APGC, Oliveira RS, Rangel AOSS, Castro PML. 2006. Zinc accumulation in *Solanum nigrum* is enhanced by different arbuscular mycorrhizal fungi. Chemosphere 65(7):1256-1263.

Moreno-Jiménez E, Gamarra R, Carpena-Ruiz RO, Millán R, Peñalosa JM, Esteban E. 2006. Mercury bioaccumulation and phytotoxicity in two wild plant species of Almaděn area. Chemosphere 63(11):1969-1973. doi:10.1016/j.chemosphere.2005.09.043.

Moreno-Jiménez E, Penalosa JM, Esteban E, Carpena RO. 2007. Mercury accumulation and resistance to mercury stress in *Rume xinduratus* and *Marrubium vulgare* grown on perlite. J. Plant Nutr. Soil Sci. 170(4): 485-494. doi:10.1002/jpln.200625238.

Ortega-Villasante C, Rellán-Alvarez R, Del Campo FF, Carpena Ruiz R O, Hernández LE. 2005. Cellular damage induced by cadmium and mercury in *Medicago sativa*. Journal of Experimental Botany 56(418):2239-2251. doi:10.1093/jxb/eri223

Patra M, Sharma A. 2000. Mercury toxicity in plants. Bot Rev. 66(3):379-422. doi:10.1007/BF02868923.

Prasad MNV. 2003. Biomarkers, in Prasad MNV, Hagemeyer J. Heavy Metal Stress in Plants. From Molecules to Ecosystem.2nd edition. Springer-Verlag, Berlin. pp. 445-448.

Rabie GH. 2005. Contribution of arbuscularmycorrhizal fungus to red kidney and wheat plants tolerance grown in heavy metal-polluted soil. Afr J Biotechnol. 4(4):332-345.

Rodríguez L, Rincón J, Asencio I, Rodríguez-Castellanos L, 2007. Capability of selected crop plants for shoot mercury accumulation from polluted soils: Phytoremediation perspectives. International Journal of Phytoremediation 9(1):1-13. doi:10.1080/15226510 601139359.

Rufyikiri G, Declerck, S, Dufey JE, Delvaux B. 2000. Arbuscular mycorrhizal fungi might alleviate aluminium toxicity in banana plants. New Phytol. 148(2):343-352. doi:10.1046/j.1469-8137.2000.00761.x.

Sierra MJ, Millán R, Esteban E, Cardona AI, Schmid T. 2008. Evaluation of mercury uptake and distribution in *Vicia sativa* L. applying two different study scales: Greenhouse conditions and lysimeter experiments. Journal of Geochemical Exploration 96(2-3):203-209. doi:10.1016/j.gexplo.2007.04.013.

Smith SE, Read DJ. 2008. *Mycorrhial Symbiosis*. Third Edition. Great Britain: Academic Press.

[US-EPA] US-Environmental Protection Agency. 2009. Potential Export of Mercury Compounds from the United States for Conversion to Elemental Mercury. Report to Congress. Washington, DC: Office of Pollution Prevention and Toxic Substances.

Wang Y, Greger M. 2004. Plant and environment interactions: Clonal differences in mercury tolerance, accumulation, and distribution in willow. J Environ Qual. 33(5):779-1785. doi:10.2134/jeq2004.1779.

World Agroforestry Centre. 2013. A tree species reference and selection guide. AgroForestryTree Database. PROSEA. http://www.worldagroforestrycentre.org/sea/ products/afdbases/af/asp/ SpeciesInfo.asp?SpID=734 [cited December 6, 2013].

Yu Y, Zhang S, Huang H. 2010. Behavior of mercury in a soil-plant system as affected by inoculation with the arbuscular mycorrhizal fungus *Glomus mosseae*. Mycorrhiza 20(6):407-414. doi:10.1007/s00572-009-0296-4.

Zornoza P, Millan R, Sierra MJ, Seco A, Esteban E. 2010. Efficiency of white lupin in the removal of mercury from contaminated soils: Soil and hydroponic experiments. J Environ Sci. 22(3):421-427. doi:10.1016 /S1001-0742(09)60124-8.