CONTRIBUTION OF ARBUSCULAR MYCORRHIZAL AND SAPROBE FUNGI TO THE TOLERANCE OF *EUCALYPTUS GLOBULUS* TO Pb

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Abstract. The application of Pb inhibited the development of mycelia of the saprobe fungi *Fusarium concolor* and *Trichoderma koningii* and the hyphal length of the arbuscular mycorrhizal fungi (AM) *Glomus mosseae* and *G. deserticola* in vitro. The application to soil of 1500 mg kg^{-1} of Pb decreases the dry weight, total N, P, Mg and Fe concentration and chlorophyll content of the shoot of *E. globulus* no inoculated with AM fungi. However, *G. deserticola* increased the dry weight, total nutrient concentration and chlorophyll content of the shoot, and the percentage of AM root length colonization and the succinate dehydrogenase activity of AM mycelia of *E. globulus* in presence of 1500 mg kg^{-1} of Pb, and these increases were higher when *G. deserticola* was inoculated together with *T. koningii*. The application to soil of 3000 mg kg^{-1} of Pb decreased the shoot dry weight and AM colonization of *E. globulus* in all treatments tested. Pb was accumulated in the stem more than in the leaves of *E. globulus*. In presence of 1500 mg kg^{-1} of Pb the highest accumulation of this metal in the stem took place when *E. globulus* was colonized with *G. deserticola*. In conclusion, the possibility to increase Lead accumulation in stem is very attractive for phytoextraction function, the saprobe fungi, AM and their interaction may have a potential role in elevating phytoextraction efficiency and stimulate plant growth under adverse conditions such as lead contaminated soil.

Keywords: arbuscular mycorrhizal fungi, phytoextraction, Eucalyptus, Pb tolerance, saprobe fungi

1. Introduction

Lead has not been shown to be essential in plant metabolism, but soil contamination with this metal can cause a diversity of damages for the plant, including loss of vegetation cover (Watanabe, 1997; McLaughlin, 2001). Evidences that several plant species can absorb and accumulate Pb from soil have been found (Baker and Walker, 1989; Huang and Cunningham, 1996; Kabata-Pendias, 2004). The use of plants to remove toxic metals from soils (phytoremediation) is emerging as a potential strategy for cost-effective and environmentally friendly remediation of contaminated soils (Cunningham and Berti, 2000). The concentrations of 100–500 mg kg^{-1} of Pb

in soils are considered to be toxic for most plants (Ross, 1994; Kabata-Pendias, 2004). However, plant sensitivity to Pb varies according to the different plant species; some of them can accumulate high concentrations of heavy metals and can be used in experimental assays for phytoremediation of contaminated soils (Huang and Cunningham, 1996; McGrath *et al.*, 2002). Many of the accumulative plants used belong to the family *Brassicaceae* but these plants produce little biomass being more interesting plant with higher biomass such as trees (Greger and Landberg, 1997). *Eucalyptus* is tree specie with a wide plasticity to grow in impoverished or marginal soils and is able to accumulate about 500 mg Pb kg^{-1} (Montoya, 1995; Pyatt, 2001).

It is known that high concentrations of Pb in soils reduce the population of soil microorganisms and their activities (Baath, 1989). Soil microorganisms play an important role in plant health, nutrient uptake and resistance against heavy metals (Kabata-Pendias, 2004). The arbuscular mycorrhizal (AM) fungi are a great component of the soil microbial biomass and it is symbiotically associated with plant roots (Brundrett *et al.*, 1996). AM fungi not only provide nutrient to the plant but also play an important role in plant tolerance to heavy metals (Fabig, 1982; Gildon and Tinker, 1983; Heggo *et al.*, 1990; Haselwandter and Berreck, 1994; Khan *et al.*, 2000; Rivera-Becerril *et al.*, 2002). Toxicity of Pb on AM fungi has been described (Barkdoll and Schenck, 1987). However, AM fungi increase tolerance of plants to Pb but the role of these fungi on Pb resistance and plant Pb uptake it is not clear (Gaur and Adholeya, 2004; Zhoug, 1999). The works majority related with trees, mycorrhizal fungi and heavy metals have been carried out with ectomycorrhizal fungi and few with AM fungi (Gaur and Adholeya, 2004; Wilkinson and Dickinson, 1995). It is known that *Eucalyptus* species are able to develop AM symbiosis and that the AM fungi *Glomus mosseae* and *G. deserticola* conferred resistance to *Eucalyptus* against Cd toxicity (Pereira, 1998; Arriagada *et al.*, 2004). Other important and common components of rhizosphere soil are the saprobe fungi (Dix and Webster, 1995). They obtain greater nutritional benefit from organics and inorganic compounds released from living roots together with sloughed cells (Alexander, 1977), can degrade toxic substances and produce AM fungal growth-stimulating substances (Madrid *et al.*, 1996; McAllister *et al.*, 1996; Fracchia *et al.*, 2000). The synergistic effect of the saprobe fungi *Fusarium concolor* and *Trichoderma koningii* on plant colonization by AM fungi and on plant resistance against heavy metals have been observed (Fracchia *et al.*, 2000; Arriagada *et al.*, 2004). However, the effect of the saprobe fungi *Fusarium concolor* and *Trichoderma koningii* on Pb uptake and Pb toxicity to plants when inoculated alone or together with the AM fungi *Glomus mosseae* and *G. deserticola* is not known. In addition, the action of the saprobe fungi on the mycorrhizal plants was very variable depending on the AM fungi and heavy metal concentration (Arriagada *et al.*, 2004; Vogel-Mikus *et al.*, 2005).

The aim of this work was to study the effects of AM, saprobe fungi and their interaction on the strategies adopted by *E. globulus* to Pb tolerance.

2. Materials and Methods

The effect of Pb on hyphal length of *Glomus mosseae* (Nicol. and Gerd.) Gerd. and Trappe (BEG no. 12) and *Glomus deserticola* (Trappe, Bloss and Menge) from Rothamsted Experimental Station was tested in 9 cm diameter Petri dishes. Sporocarps of *G. mosseae* and spores of *G. deserticola* were isolated by wet sieving the soil (Gerdemann, 1955) from alfalfa plant pot cultures and were stored in water at 4 °C. The spores of *G. mosseae*, obtained by dissecting the sporocarps, were surface-sterilized as described by Mosse (1962). Ten surface-sterilized spores per plate were placed 1 cm from the edge of a Petri dish with 10 mL of 10 mM 2-(N-morpholin) ethane sulphonic acid (MES) buffer (pH 7) plus 0.04 g of Gel-Gro (ICN Biochemicals, Aurora, OH, USA). Pb $(NO_3)_2$ was added to Petri dishes to a final concentration of 0, 50, 150, 200, 250 and 300 mg L^{-1}. Ten replicates for AM fungi were used. The plates were incubated at 25 °C in the dark for 21 days, and were sealed to reduce dehydratation and contamination. Hyphal length of the germinated *G. mosseae* and *G. deserticola* spores from five replicates was determined under a binocular microscope at 40× magnification at the end of the experiment using the gridline intersect method (Marsh, 1971). All the fungal mycelia were measured. In order to see the Pb toxicity effect on AM hyphal length was a fungistatic nature, AM spores from five replicates were transferred to new plates of Gel-Gro without Pb and the fungal mycelia were measured after 10 days of incubation.

We tested the effect of Pb on the saprobe fungi *Fusarium concolor* Schlecht. BAFC Cult. No. 2183 (Booth, 1977) and *Trichoderma koningii* Rifai (BAFC Cult. no. F8844; Rifai, 1969). These fungi were isolated from the rhizosphere soil and roots of maize cultivated in the province of Buenos Aires, Argentina by the particle washing method using a multichamber washing apparatus (Widden and Bisset, 1972). Strains are kept at the fungal culture collection of the Facultad de Ciencias Exactas y Naturales, Universidad de Buenos Aires in Buenos Aires, Argentina. Both saprobe fungi were transferred to tubes of potato dextrose agar (PDA, DIFCO) and 2% malt extract at 4 °C as stock culture. An aqueous suspension in sterile distilled water containing approximately 10^6 spores mL^{-1} of each saprobe fungus was prepared from cultures grown in potato dextrose agar (PDA, DIFCO) for 1 wk at 27 °C. Two mL of this suspension were inoculated in 250 mL flasks containing 125 mL of sterile AG liquid medium (Galvagno, 1976) in a shaker at 28 °C. The AG medium consisted in 1 g glucose, 0.4 g asparagine, 0.05 g $MgSO_2$, 0.05 K PO_2 and 100 mL distilled water. Pb $(NO_3)_2$ was added to AG medium to a final concentration of 0, 750, 1500 and 3000 mg L^{-1} Pb. After 2 weeks the number of spores per mL of culture medium was evaluated by using a Neubauer chamber (McAllister, 1992). The culture medium was filtered through a disk of filter paper, dried at 80 °C for 72 h and the dry mycelium of the saprobe fungi was weighted (McAllister, 1992). In Pb-treatment the concentration of Pb was analysed in the AG medium after 1 and 2 weeks culture of *F. concolor* and *T. koningii* (Mingorance, 2002). AG medium with 750, 1500 and 3000 mg L^{-1} Pb

but without fungal culture was used as control. Ten replicates were used in these experiments.

Eucalyptus globulus Labill. seeds previously surface-sterilised ($HgCl_2$ for 10 min) and thoroughly rinsed with sterilised water and sown in moistened sand. After germination, uniform seedlings were planted in 0.3 L pots filled with a mixture of sterilized sand:vermiculite:sepiolite (Named substrate pot) at a proportion of 1:1:1 (V:V:V). Plants were grown in a greenhouse with supplementary light provided by Sylvania incandescent and cool-white lamps, 400 E m^{-2} s^{-1}, 400–700 nm, with a 16/8 h day/night cycle at 25/19 °C and 50% relative humidity. Plants were watered from below and fed every week with 10 mL of a nutrient solution plus 50 mg L^{-1} of P (Hewitt, 1952).

The AM fungal inoculum was a root-and-soil inoculum consisting of rhizosphere soil containing spores and colonized root fragments of *Medicago sativa* L. in amounts of 8 g per pot, which were predetermined to have achieved high levels of root colonization. Uninoculated were given a filtrate (Whatman no. 1 paper) of the inoculum containing the common soil microflora, but free of AM fungal propagules.

An aqueous suspension in sterile distilled water containing approximately 10^8 spores mL^{-1} of *F. concolor* and *T. koningii* was prepared from cultures grown in potato dextrose agar (PDA, DIFCO) for 1 wk at 27 °C and 2.5 mL of this suspension were inoculated per pot.

Treatments were used: (1) Uninoculated controls (2) Substrate pot inoculated with *F. concolor* or *T. koningii* (3) Substrate pot inoculated with *G. mosseae* or *G. deserticola*, and (4) Substrate pot inoculated with *F. concolor* or *T. koningii* and either *G. mosseae* or *G. deserticola*. Plants were inoculated at the time of transplanting (after 4 weeks of growth). The saprobe fungi were inoculated at the same time as *G. mosseae* or *G. deserticola*. Five replicate per pots were used.

Pb was applied to *Eucalyptus* pots at the concentration of 0, 1500 and 3000 mg Pb Kg^{-1} of substrate pot. These concentrations were selected for showing significant toxic effect on *Eucalyptus* development (Al-Subu, 2002; Pyatt, 2001).

Plants were harvested after 12 weeks and dry mass was determined. After the harvest two samples of fresh weight were taken from the entire root system at random. One of the samples was cleared and stained (Phillips and Hayman, 1970), and the percentage of root length colonization was measured (Giovannetti and Mosse, 1980). In the second sample succinate dehydrogenase (EC 1.3.99.1) (SDH) activity was measured in fungal mycelia by the reduction of tetrazolium salts at the expense of added succinate (MacDonald and Lewis, 1978); the percentage of AM fungal mycelia with SDH activity was determined under a compound microscope (Ocampo and Barea, 1985). The total Nitrogen (N), Phosphorous (P), Potassium (K), Magnesium (Mg) and Iron (Fe) content in *Eucalyptus* plants shoots was analysed (Mingorance, 2002). To determine the total chlorophyll, the *chlorophyll a* and *chlorophyll b* of *Eucalyptus* leaves were extracted with 80% (V:V) acetone at the same developmental stage (after 12 weeks transplanting) and measured (Lichtenthaler, 1987).

Pb content in plants shoots (leaves and stems separately) was determined using the method described by Mingorance (2002).

The percentage values were arcsine transformed before statistical analysis. The data were analysed by factorial analysis of variance with AM treatment (Control, *G. mosseae* and *G. deserticola*), Saprobe fungi treatment (Control, *F. concolor* and *T. koningii*), Pb in soil treatment (0, 1500 and 3000 mg kg^{-1}) and their interaction as sources of variation.

3. Results

The results showed decreased on *G. mosseae* and *G. deserticola* hyphal length at 50 mg L^{-1} Pb treatment (Figure 1). No hyphal length was observed when higher doses than 300 mg L^{-1} of Pb were used. When spores were transferred from media with 50, 150, 200, 250 and 300 mg L^{-1} of Pb to a new Gel-Gro media without Pb, *G. mosseae* hyphal length was 15 ± 0.40; 5 ± 0.28, 2 ± 0.53, 2 ± 0.32 and 0 mm and *G. deserticola* was 18 ± 0.32, 6 ± 0.50, 4 ± 0.27, 3 ± 0.19, 1 ± 0.2 mm after 10 days of incubation.

The mycelium dry weight and spores number of *F. concolor* and *T. koningii* decrease significantly in presence of 1500 and 3000 mg L^{-1} Pb (Table I) and these concentrations decreased after culture of *F. concolor* and *T. koningii* for 1 and 2 weeks in growth media (Table II).

The results of factorial ANOVA are given in Table III. AM colonization and Pb in soil were essential for plant growth in highly Pb contaminated soil. The saprobe fungi *F. concolor* and *T. koningii* were not significant on all analyzed variables. The shoot dry weight mean for each factor and their interaction in Figure 2 illustrates that

TABLE I

Dry weight of mycelium and spores number of *Fusarium concolor* and *Trichoderma koningii* in presence of different concentration of Pb in the culture medium

Saprobe fungus	Concentration of Pb (mg L^{-1})	Dry weight of mycelium (mg)	Spores number $\times 10^5$
F. concolor	0	22.0 b	28.0 b
	750	21.2 b	25.3 b
	1500	16.1 a	18.7 a
	3000	13.4 a	15.0 a
T. koningii	0	40.0 d	36.5 c
	750	37.6 d	30.0 c
	1500	32.0 c	28.5 b
	3000	30.4 c	26.5 b

Column values followed by the same letter are not significantly different as determined by Tukey's multiple range test ($P = 0.05$).

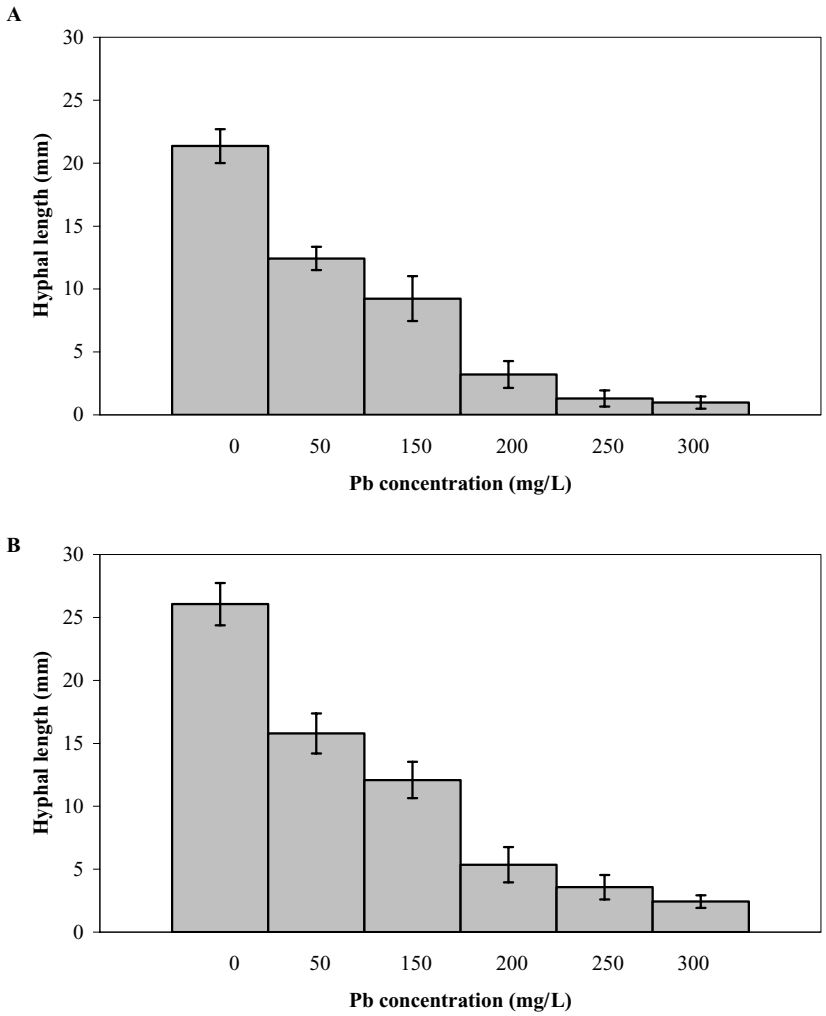

Figure 1. Effect of Pb on the hyphal length of *Glomus mosseae* (A) and *Glomus deserticola* (B) spores. The data are the means ± standard errors of means ($n = 10$).

G. mosseae did not increase the shoot dry weight of *E. globulus* but this parameter was improved by *G. deserticola* inoculated alone or together with the saprobe fungi. The application of 1500 mg Pb kg^{-1} decreased the shoot dry weight of *E. globulus* but, when *G. deserticola* and *T. koningii* were inoculated together, the shoot dry weight were significantly increased. The application of 3000 mg Pb kg^{-1} decreased the shoot dry weight of plants in all treatments tested (Figure 2 and Table III).

The AM caused the highest beneficial effect on chlorophyll content although the interaction between AM and Pb in soil was not significant (Figure 3 and Table III).

TABLE II

Pb Concentration in the growth medium inoculated with the saprobe fungi *Fusarium concolor* and *Trichoderma koningii* after one and two week culture with different Pb concentrations

	Pb concentration (mg L^{-1}) after (weeks)		
Saprobe fungus	0	1	2
F. concolor	3000	2756 ± 14.6	2606 ± 8.3
	1500	1335 ± 13.2	1247 ± 8.6
	750	608 ± 13.7	564 ± 7.9
	0	0	0
T. koningii	3000	2707 ± 12.7	2402 ± 8.9
	1500	1312 ± 13.7	1183 ± 7.3
	750	599 ± 14.0	537 ± 8.2
	0	0	0

Standard errors of means are given ($n = 10$).

TABLE III

Significance of the main treatment effects and their interactions based on factorial ANOVA

	F-values						
	AM	SF	Pb	AM × SF	AM × Pb	SF × Pb	AM × SF × Pb
Shoot dry weight	62.07***	1.85 n.s.	544.81***	6.85*	15.85***	0.65 n.s.	6.84*
Chlorophyll content	28.48***	0.23 n.s.	197.69***	0.05 n.s.	9.62***	0.20 n.s.	0.15 n.s.
Pb in stem	854.45***	1.18 n.s.	133.85***	1.38 n.s.	158.64***	0.80 n.s.	0.55 n.s.
Pb in leaf	170.67***	2.50 n.s.	191.73***	1.38 n.s.	176.51***	0.49 n.s.	0.80 n.s.

ns: not significant.
* $p < 0.05$.
** $p < 0.01$.
*** $p < 0.001$.

The effect of Pb treatment in mycorrhizal root colonization and SDH activity of *E. globulus* decreased in presence of all the doses of Pb used (Figure 4). Plants inoculated with *F. concolor* did not affect the AM root colonization and SDH activity of *Eucalyptus* in any treatments. However, dual inoculation with *G. deserticola* and *T. koningii* increased percentage of root colonization and SDH activity at 0 and 1500 mg Pb kg^{-1}. The application of 3000 mg Pb kg^{-1} decreased the percentage of root length colonization and SDH activity of *Eucalyptus* in all treatments.

G. deserticola was the only AM fungus that increased total N ($F = 7.07$; $p < 0.01$), P ($F = 8.15$; $p < 0.01$), Mg ($F = 7.48$; $p < 0.01$) and Fe concentration

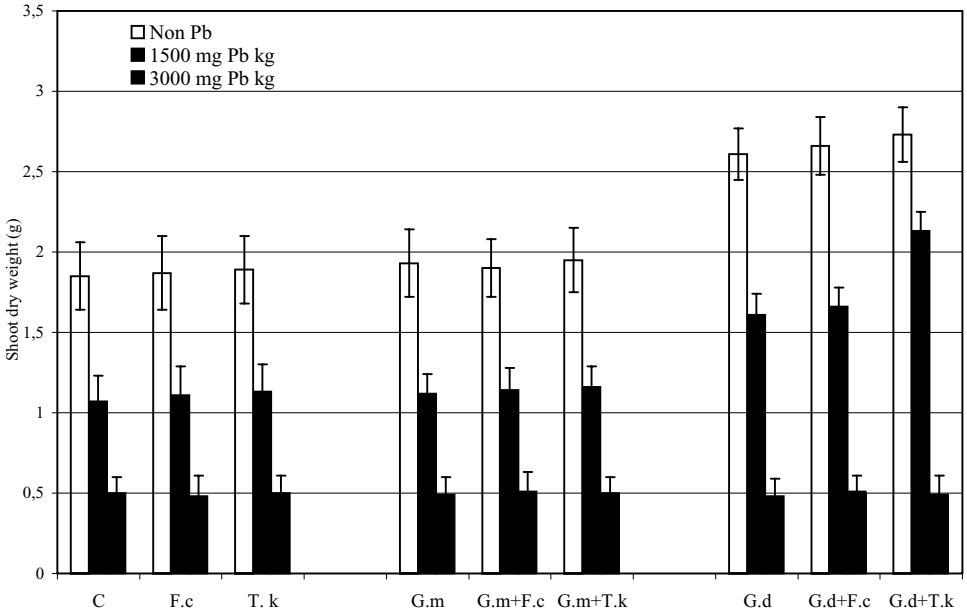

Figure 2. Shoot dry weight of *Eucalyptus globulus* inoculated or not with AM or with the saprobe fungi in soil contaminated with different Pb concentrations. C: Control; Fc: *F. concolor*; Tk: *T. koningii*; Gm: *G. mosseae*; Gm+Fc: *G. mosseae+F. concolor*; Gm+Tk: *G. mosseae+T. koningii*; Gd: *G. deserticola*; Gd+Fc: *G. deserticola+F. concolor*; Gd+Tk: *G. deserticola+T. koningii*.

($F = 15.35$; $p < 0.001$) of *Eucalyptus* shoot at 1500 mg Pb kg^{-1} (Figure 5). Dual inoculation with *T. koningii* increased the beneficial effect of *G. deserticola*. The application of 3000 mg Pb kg^{-1} decreased the total N, P, Mg and Fe concentration of *Eucalyptus* in all treatments. The K concentration in shoot plants was not significant in all interaction factors.

The effect of saprobe fungi *F. concolor* and *T. koningii* as well as the AM fungus *G. mosseae* did not influence the Pb concentration in stems and leaves of *E. globulus*. In presence of 1500 mg Pb Kg^{-1}, the concentration of this metal was higher in stems than leaves of *E. globulus* inoculated with *G. deserticola*. In presence of 3000 mg kg^{-1} similar Pb concentration in stem and leaves of *E. globulus* were observed (Figure 6 and Table I).

4. Discussion

It is known the harmful effect of Pb soil fungi (Gaad, 1993). The presence of Pb decreased the mycelial weight and the spore number of *F. concolor* and *T. koningii*. However, these saprobe fungi were able to absorb Pb from the culture medium indicating the capacity of these fungi to eliminate Pb from the medium. In fact

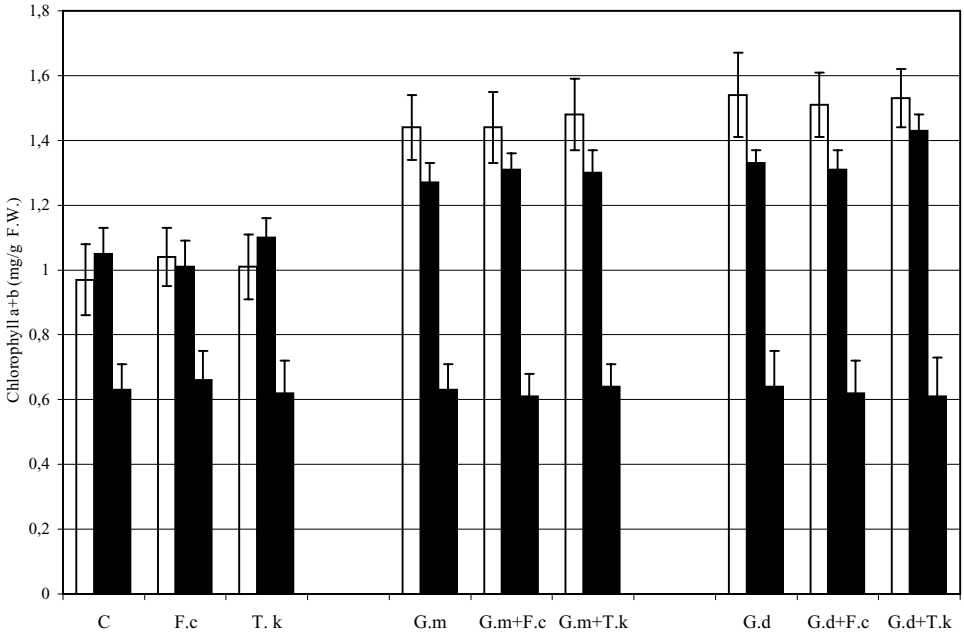

Figure 3. Chlorophyll content of *Eucalyptus globulus* inoculated or not with AM or with the saprobe fungi in soil contaminated with different Pb concentrations. C: Control; Fc: *F. concolor*; Tk: *T. koningii*; Gm: *G. mosseae*; Gm+Fc: *G. mosseae+F. concolor*; Gm+Tk: *G. mosseae+T. koningii*; Gd: *G. deserticola*; Gd+Fc: *G. deserticola+F. concolor*; Gd+Tk: *G. deserticola+T. koningii*.

some microorganism are able to absorb and to store heavy metals in their fungal structures (Huang *et al.*, 1990; Alexander, 1999; Arriagada *et al.*, 2004). Pb also inhibited the hyphal length of *G. mosseae* and *G. deserticola* spores. However, this inhibition seem to be of fungistatic nature because when these spores were transferred from media with Pb to media without Pb, they were able to develop their hyphae, although to a smaller level as compared with the spores grown in medium without Pb. These results suggest that soil contamination with high Pb concentration could decrease the development of the AM fungi in soil but, these fungi can recover their functionality when the concentrations of metal inhibitors decrease (Hepper, 1979).

The bioavailability of trace elements has been the most crucial problem in agricultural and environmental studies. High amounts of Pb in soil decrease plant growth and nutrient uptake (Fabig, 1982). There has been also described that Pb disables the biosynthesis of chlorophyll, which will produce an alteration in plant photosynthesis (Sinha *et al.*, 1993; Ouzounidou, 1995). Higher plant dry weight and N, P and Mg uptake by mycorrhizal plants compared with non-mycorrhizal in presence of Pb in soil was observed (Karagiannidis *et al.*, 1995; Bavaresco and Fogher, 1996; Andrade *et al.*, 2004). However, plant protection by AM fungi to

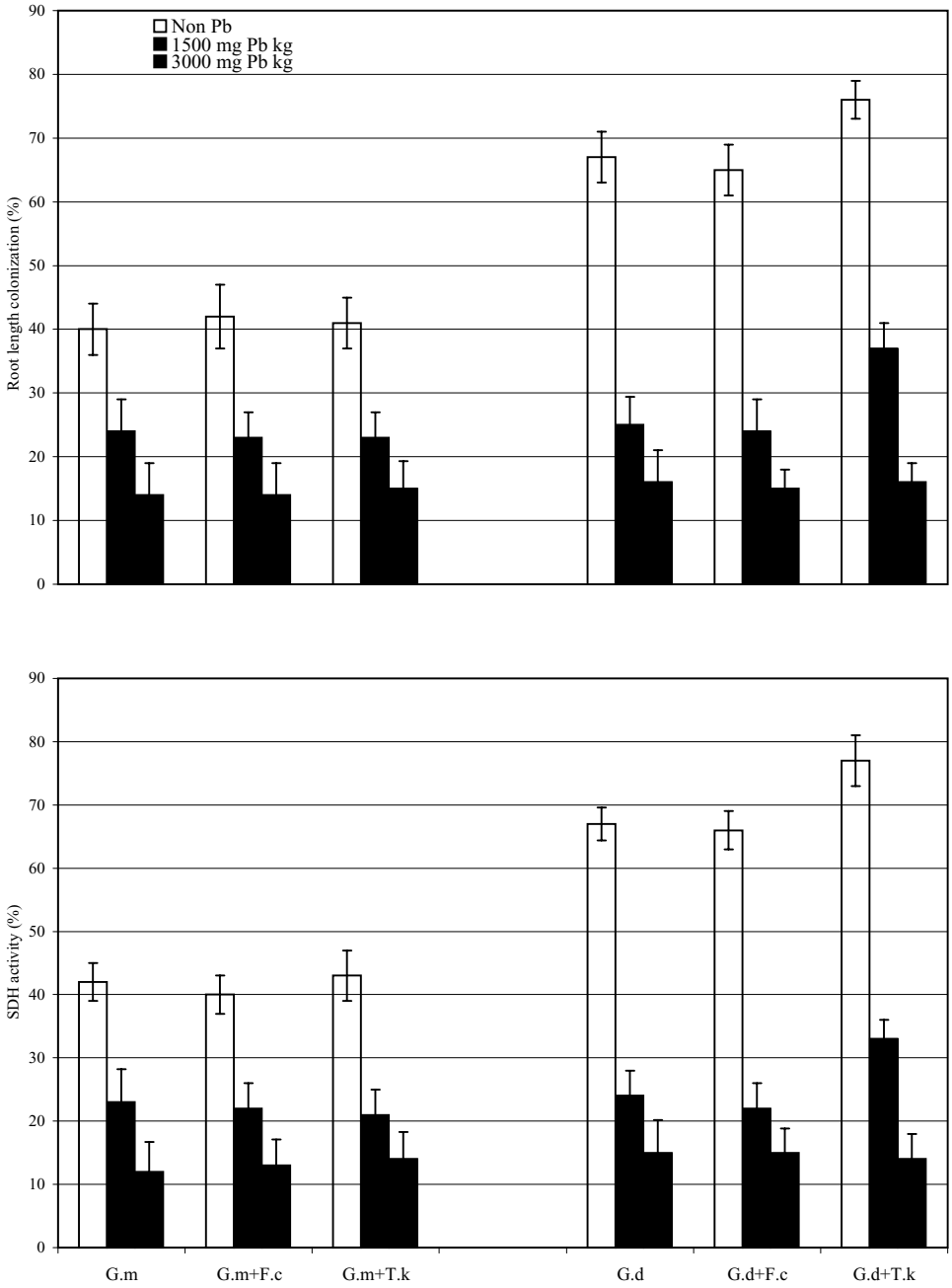

Figure 4. Effect of AM and Saprobe fungi on root length colonization and percentage of AM mycelium with SDH activity of *Eucalyptus globulus* in soil contaminated with different Pb concentrations. Gm: *G. mosseae*; Gm+Fc: *G. mosseae*+*F. concolor*; Gm+Tk: *G. mosseae*+*T. koningii*; Gd: *G. deserticola*; Gd+Fc: *G. deserticola*+*F. concolor*; Gd+Tk: *G. deserticola*+*T. koningii*.

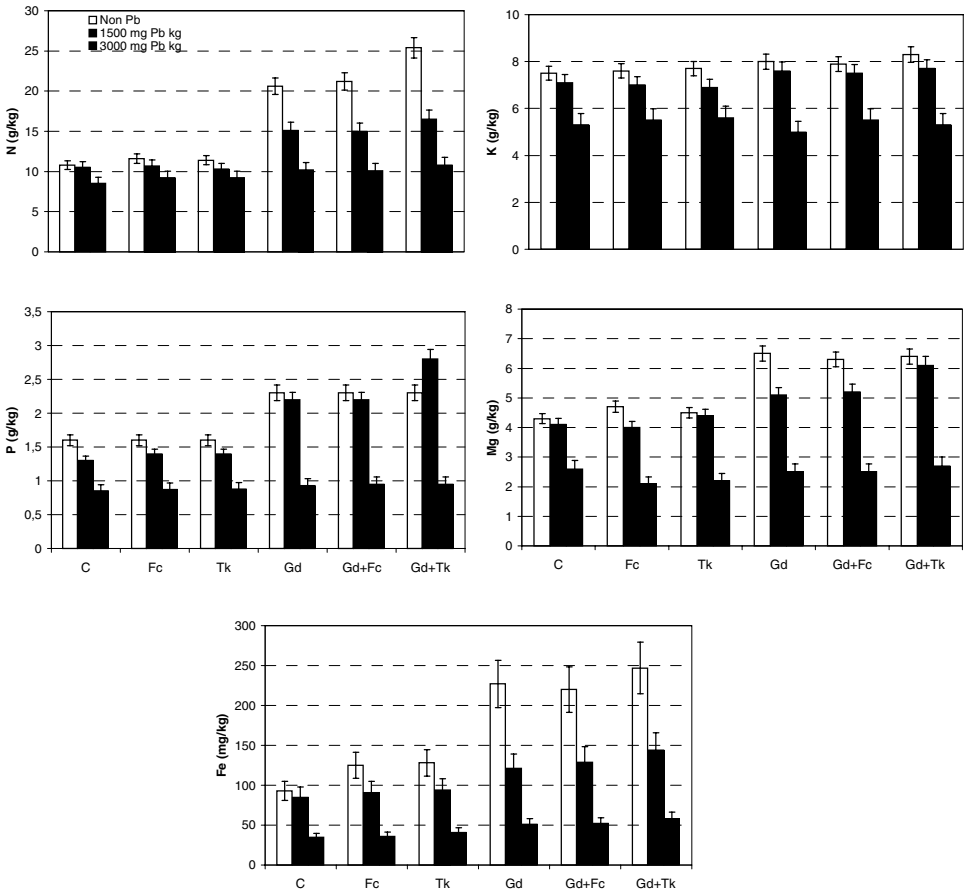

Figure 5. Mineral nutrition in shoot of *Eucalyptus globulus* inoculated or not with AM or with the saprobe fungi in soil contaminated with different Pb concentrations. C: Control; Fc: *F. concolor*; Tk: *T. koningii*; Gd: *G. deserticola*; Gd+Fc: *G. deserticola*+*F. concolor*; Gd+Tk: *G. deserticola*+*T. koningii*.

toxicity of Pb was dependent on the type of microorganism and Pb concentration (Heggo *et al.*, 1990). In fact, only *G. deserticola* increased the *E. globulus* shoot dry weight, total N, P, Mg and Fe concentration at 1500 mg Pb kg^{-1} whereas with increasing amounts of Pb in soil to 3000 mg kg^{-1} these parameters decreased. It may indicate toxic effects of Pb on the plant growth at a higher Pb concentration. In addition, studies have revealed that Pb reduces the uptake and transport of some mineral nutrients, chlorophyll content, shoot length and biomass in *Sonchus oleraceus* at the 3200 mg Pb kg^{-1} (Xiong, 1997). The mycorrhizal plants showed increase in P uptake compared with corresponding non-mycorrhizal plants under metal contaminated soil (Chen *et al.*, 2003; Karagiannidis and Nikolaou, 2000). The improved P nutrition might be a mechanism involved in the alleviation of Pb

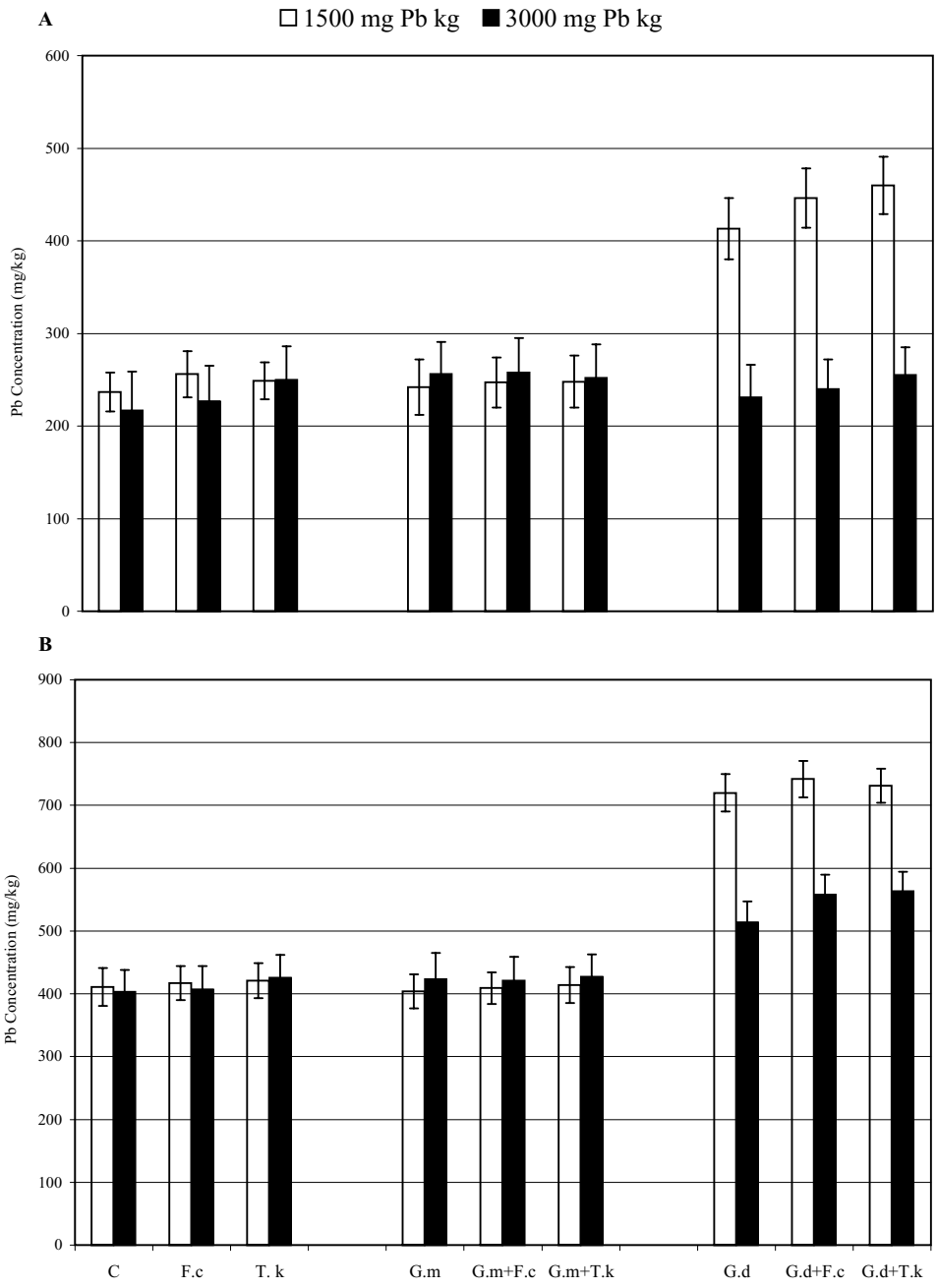

Figure 6. Pb concentration in leaves (A) and stem (B) of *Eucalyptus globulus* inoculated or not with AM or with the saprobe fungi in soil contaminated with different Pb concentrations. C: Control; Fc: *F. concolor*; Tk: *T. koningii*; Gm: *G. mosseae*; Gm+Fc: *G. mosseae*+*F. concolor*; Gm+Tk: *G. mosseae*+*T. koningii*; Gd: *G. deserticola*; Gd+Fc: *G. deserticola*+*F. concolor*; Gd+Tk: *G. deserticola*+*T. koningii*.

toxicity as a result of mycorrhizal colonization. The increase in Mg and Fe synthesis in plant inoculated with *G. deserticola* alone or together with *T. koningii* could have contribute to the increase the total chlorophyll synthesis (Cordeiro *et al*., 1995). In fact, the major production of total chlorophyll by *E. globulus* colonized with AM fungi indicates that were more efficient in the absorption of light, which will affect the photosynthetic efficiency of the plants (Gil, 1995). These effect induced by saprobe and AM fungi should be taken into consideration when studying the effect of heavy metals on Fe and Mg deficiency.

Synergistic action of saprobe fungi belonging to *Fusarium* and *Trichoderma* genera on the AM colonization of root has been observed (Fracchia *et al.*, 1998; Garcia-Romera *et al.*, 1998). The fact that saprobe fungi can absorb Pb and that some of them can increase AM colonization of plant may explain that the combined inoculation of *G. deserticola* and *T. koningii* increased the tolerance of *E. globulus* to the application of 1500 mg Pb kg^{-1}. Nevertheless, when 3000 mg Pb kg^{-1} was applied the protective effect of *G. deserticola* and *T. koningii* disappeared. Possibly Pb uptake by AM and saprobe fungi was not sufficient to decrease the plant toxicity at 3000 mg Pb kg^{-1} probably by reduce the AM root length colonization and metabolic activity of *G. deserticola*. These results indicated that the presence of high Pb concentration in soil also decreased the development of the AM fungus inside the root and decreased its contribution to the Pb accumulation in the plant. On the other hand, the saprobe fungi, inoculated alone did not decrease the toxic action of Pb on *E. globulus*. This indicates that the beneficial effect of *T. koningii* was attributable to its synergistic effect on root colonization by *G. deserticola* more than Pb uptake.

Accumulation and exclusion are two basic strategies by which plants respond to elevated concentrations of heavy metals. The concentrations of 100–500 mg Pb kg^{-1} are considered to be toxic for most plants (Ross, 1994; Levy *et al.*, 1999; Kabata-Pendias, 2004). However, some *Eucalyptus* species were tolerant to concentration of 500 mg Pb kg^{-1} (Pyatt, 2001). Thresholds for plant hyperaccumulation (shoot dry weight) were set at 1000 mg kg^{-1} (0.1%) Pb (Reeves and Baker, 2000). Many plants increase their tolerance to heavy metals by transferring small amounts of these metals to the shoot (Cunningham *et al.*, 1995; Arriagada, 2004). Our results show that about 67% of Pb was accumulated in the stem more than in the leaves of *E. globulus* especially when 1500 mg Pb kg^{-1} was applied. Shoot Pb concentrations exceeded the hyperaccumulation criterion with a maximal concentration of 1191 mg kg^{-1} and suggested consider an *E. globulus* possible hyperaccumulating Pb by beneficial interaction between AM and saprobe fungi. The higher Pb accumulation in less metabolically active part of plant indicates that the damage caused on the plant physiology will be minor (Leep and Dickinson, 1998). The AM fungi seem to contribute an increased heavy metal uptake by plant (Andrade *et al.*, 2004). In our experiment higher accumulation of Pb in stem that in leaves of *E. globulus* when colonized with *G. deserticola* at 1500 mg Pb kg^{-1} was observed. The main Pb concentration takes place in the stem, where the harmful

effects on the plant development are minor, can explain why *G. deserticola* increased the resistance of *E. globulus* to Pb toxicity in spite of high Pb accumulation in plant shoot. In conclusion, the possibility to increase Lead accumulation in stem by *E. globulus* is very attractive for phytoextraction function, the saprobe fungi, AM and their interaction may have a potential role in elevating phytoextraction efficiency and stimulate plant growth under adverse conditions such as lead contaminated soil.

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