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Journal of Biotechnology 116 (2005) 369–378
JOURNAL OF Biotechnology
www.elsevier.com/locate/jbiotec

Application of *Aspergillus niger*-treated agrowaste residue and *Glomus mosseae* for improving growth and nutrition of *Trifolium repens* in a Cd-contaminated soil

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Received 3 May 2004; received in revised form 15 December 2004; accepted 20 December 2004

Abstract

The microbial transformation of sugar beet (SB) agrowaste with or without rock-phosphate (RP) has utility for the improvement of plant growth in a Cd (5 $\mu g\, g^{-1}$) artificially contaminated soil, particularly when the soil is co-inoculated with arbuscular mycorrhizal (AM) fungus *Glomus mosseae* isolated from a Cd-polluted area. Under such Cd-polluted conditions, the limited growth, mineral nutrition, symbiotic developments (nodulation and AM-colonization) and soil enzymatic activities were stimulated using SB or SB + RP as soil amendments and *G. mosseae* as inoculant. *G. mosseae* enhanced plant establishment in a higher extent in amended soil; it is probably due to the interactive effect increasing the potential fertility of such compounds and its ability for decreasing Cd transfer from soil to plant. The amount of Cd transferred from soil solution to biomass of AM-colonized plants ranged from 0.09 $\mu g\, Cd\, g^{-1}$ (in SB + RP-amended soil) to 0.6 $\mu g\, Cd\, g^{-1}$ (in non-amended soil). Nodule formation was more sensitive to Cd than AM-colonization, and both symbioses were stimulated in amended soils. Not only AM-colonization but also amendments were critical for plant growth and nutrition in Cd-polluted soil. The high effectiveness of AM inoculum increasing nutrients and decreasing Cd in amended soil indicated the positive interaction of these treatments in increasing plant tolerance to Cd contamination.

Keywords: Amendments; Cd contamination; *Glomus mosseae*; Rhizobium

1. Introduction

Elevated concentrations of heavy metals in soil from anthropogenic sources (fertilizers, amendments) or mining activities (Shetty et al., 1995) poses long-term risk to environmental and sustainable production.

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doi:10.1016/j.jbiotec.2004.12.009

The deteriorated physical and biological characteristics of contaminated soils need to be improved to establish a vegetation cover. To reach the objectives based on recovering soil quality properties, the application of transformed organic agrowaste residues has interest since it is a meaning of increasing soil organic matter content and improving physical–biological properties (Medina et al., 2004a,b; Caravaca et al., 2004).

The toxicity of metals in soil depends on their bioavailability that is considered as the ability to be transferred from the soil solution to the plants growing on it. Nevertheless, metal bioavailability is not only dependent on metal concentration in the soil; factors such as organic matter and soil microorganisms are involved, as well.

In degraded soil, the activity of microbiota is low because of the lack of suitable organic substrates. The lack of availability of simple sugars normally limits the development of heterotrophic organisms. Nevertheless, this is not a limiting step if agrowaste amendments are incorporated into the unfertile soil. Soil enzymes play an important role in the mineralization of organic products, making fundamental ions available for plant growth (Díaz-Raviña et al., 1992). The microbial activity is of great importance in nutrient cycling and energy flow, and Alexander (1967) determined that microbial metabolism releases 1–4% of the soil organic N in available form for plants during the growth period.

Microbial metal tolerance has been demonstrated and it has interest from an ecological and practical point of view. Mycorrhizal fungi able to colonize polluted soil suggest the metal tolerance of these fungi. But in polluted soils the mycorrhizal component may disappear or, at least, be severely depleted (Bååth, 1989).

There is evidence that mycorrhizal fungi help the plant to thrive under polluted conditions (Shetty et al., 1994a,b). The fungi involved in arbuscular mycorrhizal (AM)-colonization provide a direct link between soil and root, which increases the supply of nutrients to the plant.

Some AM fungi have the capacity to decrease Cd concentration in plant growing in Cd-polluted soil (Joner et al., 2000; Leyval et al., 1997; Vivas et al., 2003a,b). These findings corroborate that soil fungi and bacteria represent important soil components of Cd immobilization (Speir et al., 1999). On the other hand, organic matter addition, as amendment, can improve AM development (Douds et al., 1997; Gaur and Adholeya, 2000) and metal sequestration (Oudeh et al., 2002). Thus, it can be considered as an alternative strategy to prevent Cd toxicity.

The use of sugar beet (SB) agrowaste of lignocellulosic composition as a amendment has been recommended after microbial treatment with *Aspergillus niger* in medium added of rock-phosphate (RP) (Rodríguez et al., 1999; Vassilev et al., 1996). *A. niger* grown on SB residue was able to solubilize RP by its ability to excrete organic acids (citric acid mainly). As a result of such fermentative process, RP is solubilized and lignocellulolitic (SB) material is transformed in more simple sugars compounds. The application of such product to soil as amendment was effective in improving physical, chemical and microbiological properties of soil (Caravaca et al., 2004). Nevertheless, no information exists on the use of such materials in a bioremediation program.

In this study, we used an artificially Cd-contaminated soil, for assessing the effectiveness of *A. niger*-treated SB in presence and in absence of RP, in interaction with an Cd-adapted *Glomus mosseae* strain, in reducing Cd plant toxicity and in promoting plant establishment. Biological characteristics of amended and non-amended Cd-polluted soils were determined in presence or absence of AM-colonization.

2. Materials and methods

2.1. Fermentation process

NB2 strain of *A. niger* was used in this study. It had previously been selected as producing citric acid on complex substrates (Vassilev et al., 1986).

Sugar beet waste, a lignocellulosic material, was ground in an electrical grinder to 1 mm fragments. It was mixed at a concentration of 10% with 50 ml Czapek's solution (described in Fluka Chemica, catalogue no. 70185) contained ($g\,l^{-1}$ of distilled water): $FeSO_4$, 0.01; $MgSO_4{\cdot}7H_2O$, 0.5; KCl, 0.5; $NaNO_3$, 3.0; sucrose, 30; K_2HPO_4, 1.0 and a final pH of 7.3 ± 0.2 for static fermentation in 250 ml Erlenmeyer flasks. The latter were inoculated with 3 ml of *A. niger* spore suspension (1.2×10^6 spores). RP at a concentration of $1.5\,g\,l^{-1}$ was added when appropriate. Static fermentation was performed at 28 °C for 20 days.

2.2. Soil–plant experiment

The experiment consisted of a completely randomized factorial block with two factors: (1) mycorrhizal inoculation with autochtonous *G. mosseae* strains and (2) *A. niger*-treated SB amendment supplemented or not with RP before fermentation process. Non-SB-treated control was also used. All treatments were replicated five times with a total of 30 pots.

The soil used for the experiment was from the Granada province (Spain), with pH 7.2 (water), 1.6% organic matter, nutrient concentrations: 2.1 mg kg^{-1} N; 1.7 mg kg^{-1} P ($NaHCO_3$-extractable P); 0.8 mg kg^{-1} K. The soil texture was made up of 57.8% sand, 19% clay and 23.2% silt. The soil was air-dried, sieved to less than 2 mm, mixed with quartz-sand (<1 mm) at a ratio of soil:sand of 2:1 (v/v) and sterilized by steaming for three sterilization cycles (100 °C for 1 h for 3 days). After sterilization, the soil was supplemented with Cd by adding adequate amount of an aqueous solution of $CdSO_4$. After 2 weeks of incubation (for metal stabilization), the available amount of Cd was 5 μg g^{-1} determined using EDTA as extractant (Lakanen and Erviö, 1971).

The amendment was mixed (when necessary) at a rate of 5% with the soil–sand mixture and left for equilibration for 3 weeks at room temperature.

Rock-phosphate (0.75 g per pot) was applied to the pots that had not received the RP-supplemented amendment.

Ten seeds of *Trifolium repens* were planted in each pot (500 ml capacity) inoculated or not with indigenous AM inoculum of a Cd-adapted *G. mosseae* strain, isolated from Cd-treated long-term field experiment (10 years old) at Nagyhorcsok (Hungary) (Vörös et al., 1998).

The *G. mosseae* strain was bulked in a open-pot culture of red clover and consisted of spores, mycelium and mycorrhizal root fragments. Ten grams of inoculum were applied to each one of the corresponding pots in the bottom of a 5-cm-deep hole. The seedlings were thinned to 4 per pot, 2 weeks after emergence.

Rhizobial inoculum consisted in a suspension (1 ml, 10^8 cfu per pot) of *Rhizobium leguminosarum* var *trifolii* prepared following standard procedure.

Non-mycorrhizal treatments received the same amount of autoclaved inoculum and a filtrate (2 ml) of AM inoculum in order to add the microbial population free of AM propagules.

The plants were grown in a greenhouse under a day/night cycle of 16–8 h, 21–15 °C and 50% relative humidity. Photosynthetic photon flux density (PPFD) was 500 μmol m^{-2} s^{-1} as measured with a light meter (LICOR, model LI-188B). Water loss from field capacity was replaced daily by top watering (tap water).

2.3. Determination of growth and symbiotic parameters

After 3 months, plants were harvested and the dry biomass of shoots, and roots, nutrients and metals concentrations in shoots, and symbiotic development (mycorrhizal infection and nodulation) were determined. Shoot concentrations (mg g^{-1}) of N (micro-Kjeldahl) K and P, as well as of Cd (μg g^{-1}) were determined. Three different measurements made on a pooled sample containing the five replicate pots per treatment. Analyses were clone after wet digestion of the air-dried plant samples with $HNO_3 + H_2O_2$ by atomic absorption spectrometer (A.A.S.) model 5000 of Perkin-Elmer, Germany.

Roots were carefully washed and then divided into two batches: one was stained by the classical non-vital trypan blue (TB) staining (Phillips and Hayman, 1970) and the other was used for histochemical vital staining succinate dehydrogenase (SDH) in order to measure total (TB) and living (SDH) AM fungal development.

Succinate dehydrogenase activity was revealed according to the procedure described by Smith and Gianinazzi-Pearson (1990). The roots were immersed in a freshly made solution containing 0.2 M Tris–HCl pH 7.0, 2.5 M sodium-succinate hexahydrate, 4 mg ml^{-1} nitro blue tetrazolium, 5 mM $MgCl_2$. Root fragments were stained overnight at room temperature and then cleared for 15–20 min in a 3% active chlorine solution of sodium hypochlorite.

Mycorrhizal development, after either non-vital or vital staining procedures, was evaluated by the method of Trouvelot et al. (1986) (for more information, see http://www.dijon.inra.fr/bbceipm/Mychintec/Mycocalc-prg/). An estimate of the length of root colonized by the fungus (the colonization frequency, *F*%) is given as the ratio between colonized root fragments and the total number of root fragments observed. The colonization intensity (*m*%) is an estimate of the amount

of root cortex that became mycorrhizal, relative only to the mycorrhizal root fraction, while *M*% is the colonization intensity relative to the whole root system. Arbuscule abundance (*a*% and *A*%) is an estimate of arbuscule richness in the mycorrhizal root fraction and in the whole root system, respectively.

Nodule formation was determined in clean roots by direct observation using a binocular microscope.

2.4. Soil analyses

In rhizospheric soil samples, enzymatic activities as well as indole acetic acid (IAA) production were determined.

Dehydrogenase activity was determined following Skujins' method (1976), as modified by García et al. (1997). For this, 1 g of soil at 60% of its field capacity was exposed to 0.2 ml of 0.4% INT (2-*p*-iodophenyl-3-*p*-nitrophenyl-5-phenyltetrazolium chloride) in distilled water for 20 h, at 22 °C in darkness. The iodonitrotetrazolium formazan (INTF) formed was extracted with 10 ml of methanol, by shaking vigorously for 1 min and filtering through a Whatman No. 5 filter paper. INTF was measured spectrophotometrically at 490 nm.

Acid phosphatase activity was determined using *p*-nitrophenyl phosphate disodium (PNPP, 0.115 M) as substrate. Two milliliters of 0.5 M sodium acetate buffer adjusted to pH 5.5 using acetic acid (Naseby and Lynch, 1997) and 0.5 ml of substrate were added to 0.5 g of soil and incubated at 37 °C for 90 min. The reaction was stopped by cooling at 2 °C for 15 min. Then, 0.5 ml of 0.5 M $CaCl_2$ and 2 ml of 0.5 M NaOH were added, and the mixture was centrifuged at 4000 rpm (1780 × *g*) for 5 min. The *p*-nitrophenol (PNP) formed was determined in a spectrophotometer (Turner Model 350) at 398 nm (Tabatabai and Bremner, 1969). Controls were made in the same way, although the substrate was added before the $CaCl_2$ and NaOH.

β-Glucosidase was determined using *p*-nitrophenyl-β-D-glucopyranoside (PNG, 0.05 M; Masciandaro et al., 1994) as substrate. This assay is also based on the release and detection of PNP. Two milliliters of 0.1 M maleate buffer (pH 6.5) and 0.5 ml of substrate were added to 0.5 g of sample and incubated at 37 °C for 90 min. The reaction was stopped with tris-hydroxymethyl aminomethane (THAM) according to Tabatabai (1982). The amount of PNP was determined in a spectrophotometer at 398 nm (Tabatabai and Bremner, 1969).

The indole acetic acid in rhizosphere soil was determined by a colorimetric method developed by Mitchell and Brunstetter (1939) and Gordon and Weber (1950). For that, 2.0 g of air-dried soil (on an oven-dry basis) were place in a 50 ml Erlenmeyer flask and 6 ml of phosphate buffer (pH 7.5) with glucose (1 g glucose/100 ml phosphate buffer) and 4 ml of L-tryptophan (1 g tryptophan/100 ml H_2O) were added. These soil solutions were mixed, stoppered and incubated at 37 °C for 24 h in the dark. For the extraction, 2 ml of 5% trichloroacetic acid solution were added to inactivate the enzymes involved in the bioassay of auxin, then 1 ml of 0.5 M $CaCl_2$ solution were added. The soil solution was filtered (Whatman No. 2). Three milliliters of the filtrate were put in a test tube, and to this 2 ml of salper solution (2 ml 0.5 M $FeCl_3$ and 98 ml 35% perchloric acid) were added. This mixture was incubated for 30 min at 25 °C in the dark. Then, the absorbance of the red solution was measured with a spectrophotometer adjusted to a wavelength of 535 nm (Wöhler, 1997).

2.5. Statistics

The results (except concentration and content of elements on shoot) were statistically evaluated by factorial analysis of variance with bacterial treatment, mycorrhizal treatment and bacterial treatment–mycorrhizal treatment interaction as sources of variation. Percentage values were arcsine-transformed before statistical analysis.

3. Results

Trifolium plants failed to establish when Cd-contaminated soil was non-amended and lacking of AM inoculum (Fig. 1).

The application of amendments and AM inoculation significantly increased shoot biomass at whatever harvest time. SB + RP amendment was the most effective treatment increasing plant growth in absence of AM-colonization while AM-colonized plants reached the highest growth in SB-amended soil (Fig. 1).

In non-amended Cd-polluted soil, plant growth was seriously limited but AM-colonization greatly

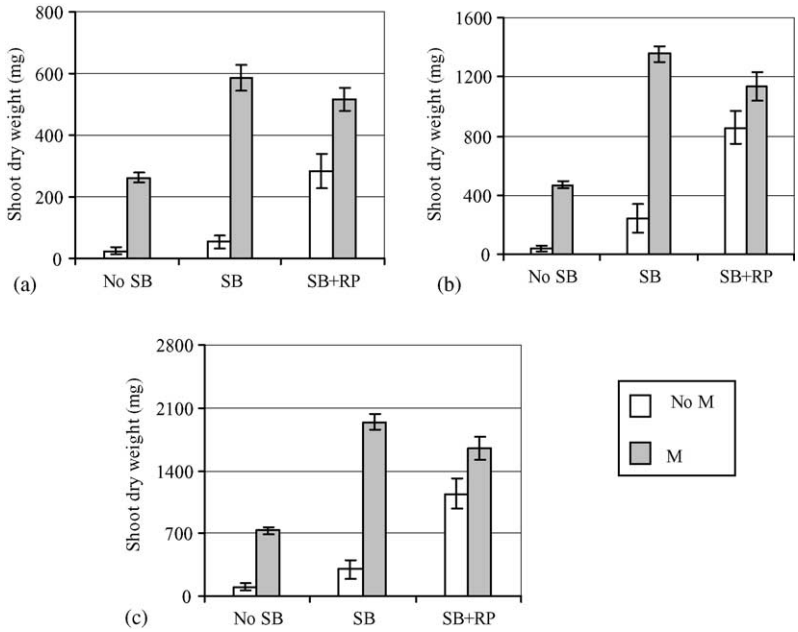

Fig. 1. Effect of amendments (SB or SB + RP) and mycorrhizal inoculation (M) on shoot dry biomass [first (a), second (b) and total (c) harvests] of *Trifolium* plants growing in Cd (5 $\mu g\, g^{-1}$)-contaminated soil. Verticals bars represent standard errors.

improved plant biomass. In the first harvest SB + RP amendment affected plant growth in a similar extent than AM inoculum (Fig. 1a); but in the second harvest, the amendment (SB + RP) had a higher effectiveness in increasing shoot biomass than *G. mosseae* colonization without amendment (Fig. 1b). The mycorrhizal colonization in amended soil increased shoot growth by 1089% (SB) and by 192% (SB + RP) over non-treated plants. These results show that not only AM-colonization but also SB amendment were critical for plant growth in Cd-polluted soil. Mycorrhizal responses were different depending on the amendment applied. AM-colonization was particularly effective increasing shoot and root growth in soil amended with SB (Figs. 1 and 2).

The effect of AM fungus on root development was particularly marked in SB-amended soil which resulted the best treatment for enhancing such value; this was

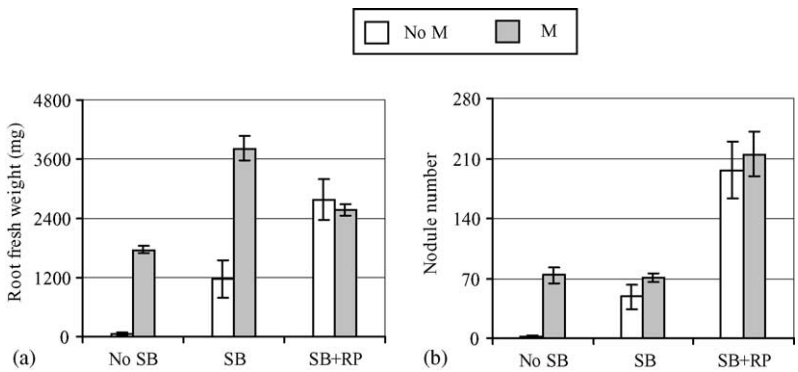

Fig. 2. Effect of amendments (SB or SB + RP) and mycorrhizal inoculation (M) on root fresh weight (a) and nodule numbers (b) formed in Cd (5 $\mu g\, g^{-1}$)-contaminated soil. Verticals bars represent standard errors.

more important on root growth than on nodule production (Fig. 2a and b). While root growth was strongly increased by amendments or AM-colonization (control or SB-treated plants) the nodule number did not respond to AM inoculum in SB or SB + RP-amended soil (Fig. 2b). Nodule number was nearly zero in non-mycorrhizal non-amended plants (Fig. 2).

Regarding plant nutrient acquisition, AM-colonization or SB amendments increased shoot P concentration. The highest P concentration was analyzed in AM inoculated plants growing in SB + RP-amended soil (Fig. 3b). In contrast, the lowest Cd concentration in shoot tissues was observed in mycorrhizal plants growing in SB + RP-amended soil (Fig. 3a). Mycorrhizal colonization was particularly effective increasing P and decreasing plant Cd concentration in SB + RP-amended soil. In mycorrhizal plants, Cd transferred from soil to plant biomass ranged from 0.09 $\mu g\,Cd\,g^{-1}$ (SB + RP-amended soil) to 0.6 $\mu g\,Cd\,g^{-1}$ (non-amended soil). These results are a confirmation of the effective interaction of these treatments (AM inoculation and SB amendments) improving nutrients and decreasing Cd in shoot tissues resulting in an increased plant tolerance to Cd contamination.

There were significant differences in foliar N and K content between mycorrhizal plants growing in non-amended or growing associated with each one of the amendments (Fig. 4a and b). The highest N or K plant acquisition were observed in AM-colonized plants but the interaction of *G. mosseae* with SB was more effective in enhancing N and K content than with SB + RP. Nevertheless, SB amendment increased N and K content in a lesser extent than SB + RP (Fig. 4).

With regard to AM-colonization (Table 1), all the inoculated plants, irrespective of amendments, showed a high frequency (%*F*) of colonization and nearly the totality remained alive (SDH staining). The intensity of mycorrhizal colonization (%*M* and %*m*) increased as affected by amendments, but colonization intensity was particularly stimulated by SB. Nevertheless, the highest differences between treatments were found regarding the arbuscule richness (%*a* and %*A*). Arbuscule formation was totally inhibited in absence of amendments. But the detrimental effect of Cd on arbuscule formation seems to be compensated by amendments, particularly SB + RP. The arbuscule richness in the mycorrhizal root fraction (%*a*) estimated after the vital staining (SDH) was highly stimulated in SB + RP-amended soil, while %*A* was similar for the two amendments.

Amended soil showed higher enzymatic activities in the rhizosphere than non-amended soils (Fig. 5). Some changes on these hydrolytic activities were also induced by AM-colonization. The strongest effect of amendments application was observed on β-glucosidase activity that was very low in rhizosphere of non-amended soil (Fig. 5b). The highest dehydrogenase and phosphatase activities were tested in mycorrhizal and SB + RP-amended soils (Fig. 5a).

Amendments increased IAA production in rhizosphere soil more than AM-colonization (Fig. 5d).

4. Discussion

A relevant effect of amendments was the improvement of the arbuscular colonization, which was not observed in highly colonized plants in absence of amendment. This effect may contribute to a higher performance of AM-colonized plants in Cd-polluted soil.

No previous information of the influence of SB or SB + RP amendments and AM-colonization on plant Cd tolerance has been reported. The effectiveness of AM-colonization, using a Cd-adapted *G. mosseae* strain, particularly when associated to SB amendment is demonstrated in this study. SB + RP amendment was the most efficient in decreasing Cd concentration in shoot tissue of AM plants, probably due to the fact that P concentration in the medium was the highest when RP was applied together with SB at the fermentation process. The phytotoxicity caused by Cd seems to be alleviated by a minor absorption of Cd and to an indirect effect of increased P nutrition (Sieverding, 1991).

If Cd bioavailability in the experimental soil is regarded as the amount of Cd transferred from the soil solution (5 $\mu g\,g^{-1}$) to the plant growing in such soil, it was reduced by the treatments applied. In SB + RP-amended soil, only 0.09 $\mu g\,Cd\,g^{-1}$ was transferred from the soil to mycorrhizal plant biomass while in non-amended soil the Cd amount transferred to the shoot of mycorrhizal plants was 0.6 $\mu g\,Cd\,g^{-1}$. The combination of SB + RP and *G. mosseae* highly decreased Cd bioability. Thus, plant Cd toxicity resulted to be more related with the treatment applied than with the Cd concentration in the soil.

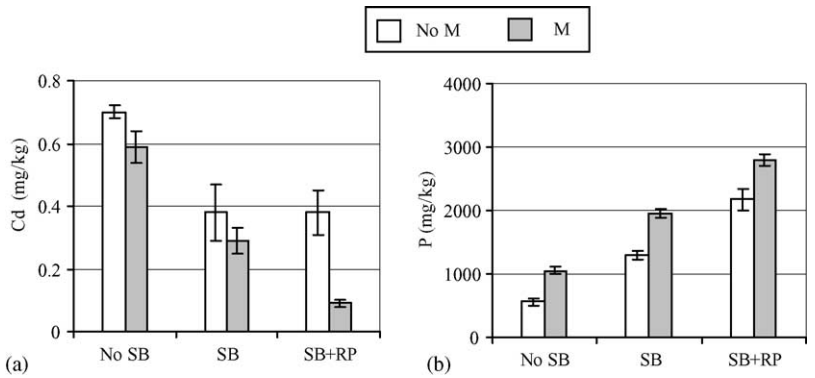

Fig. 3. Effect of amendments (SB or SB + RP) and mycorrhizal inoculation (M) on Cd and P concentration (a and b, respectively) in shoot tissue of *Trifolium* plants growing in Cd (5 $\mu g\, g^{-1}$)-contaminated soil. Verticals bars represent standard errors.

The effect of SB + RP amendment in addition to AM-colonization decreasing Cd transfer from soil to plant can be regarded as an interesting mechanism for alleviating metal toxicity in plants. AM-colonization is able to decrease Cd concentration in plants growing in Cd-polluted soil (Joner et al., 2000) and the amendment itself, apart from the metal sequestration ability, improved AM development and function. The dual application of these treatments resulted an efficient strategy capable of removing Cd from contaminated soil and thus of preventing Cd toxicity in plant.

The absence of nodule in non-mycorrhizal and non-amended plants indicate that inoculated *Rhizobium* was highly sensitive to the high available Cd concentration in soil (Biró et al., 1995) and it was not able to colonize roots in non-treated soil. But this detrimental effect of Cd on nodule formation was compensated by amendments or AM-colonization. The highest nodule production in SB + RP added medium may be due to the greatest P amount in this amendments as result of RP solubilizing ability of *A. niger* on this cellulosic substrate (Rodríguez et al., 1999; Vassilev et al., 1996).

The AM-colonized plants grown in amended soils showed the highest percentages of AM-colonization in terms of frequency (%*F*). Nevertheless, infective parameter as %*m* or %*M* and, in a higher extent, %*a* or %*A* showed a high sensitivity to the Cd-polluted conditions used. In unamended soils, intracellular mycelium development (%*m* or %*M*) was highly reduced and no arbuscules (%*a* and %*A*) were formed. The importance of arbuscules in the exchange of nutrients between the plant and the AM fungus is determinant in the symbiotic relationship. Thus, the important role of SB + RP

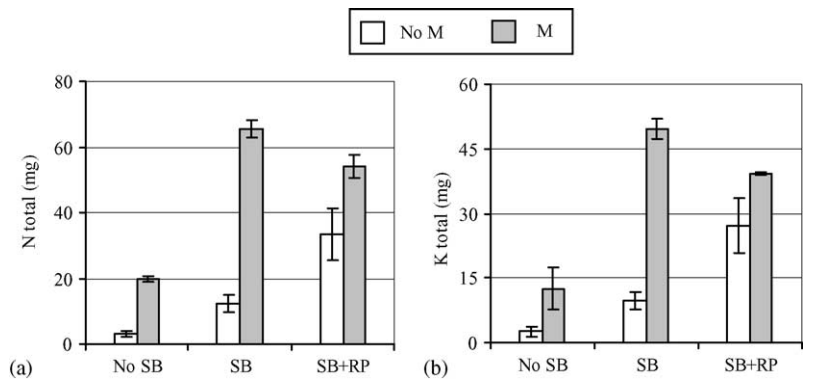

Fig. 4. Effect of amendments (SB or SB + RP) and mycorrhizal inoculation (M) on N and K content (mg) (a and b, respectively) in shoot tissue of *Trifolium* plants growing in Cd (5 $\mu g\, g^{-1}$)-contaminated soil. Verticals bars represent standard errors.

Table 1
Effect of amendments (SB or SB + RP) on AM-colonization observed as trypan blue (TB) and succinate dehydrogenase (SDH) staining in roots of *Trifolium* plants growing in Cd (5 μg g^{-1})-contaminated soil

	TB staining			SDH staining		
	No SB	SB	SB + RP	No SB	SB	SB + RP
%*F*	91 ± 2.90	100 ± 0.00	100 ± 0.00	88 ± 6.72	100 ± 0.00	90 ± 5.22
%*M*	25 ± 4.94	65 ± 6.44	50 ± 5.34	18 ± 3.39	43 ± 12.70	30 ± 7.19
%*m*	27 ± 5.38	65 ± 6.44	50 ± 5.35	20 ± 3.80	43 ± 12.70	32 ± 6.96
%*a*	0 ± 0.00	19 ± 5.07	36 ± 5.35	0 ± 0.03	7 ± 3.98	22 ± 9.71
%*A*	0 ± 0.00	12 ± 3.98	19 ± 4.28	0 ± 0.00	4 ± 2.82	4 ± 1.63

Mycorrhizal values were—%*F*: percentage of root fragments with fungal colonization, %*M*: percentage of the fractional colonization extent in the root system, %*m*: percentage of colonization in the root fraction of 1 cm, %*a*: percentage of arbuscule abundance in the root fraction of 1 cm, %*A*: percentage of root cortex with arbuscules. Standard errors of the means are given.

increasing arbuscule colonization and its vitality in the root fraction (%*a*).

Surprisingly, the highest symbiotic developments (nodule formation or AM-colonization) on SB + RP-amended soil did not result in the highest N plant content or in the greatest plant biomass. This lack of correspondence must be due to some nutrient disequilibria here not determined.

The inoculation with *G. mosseae* increased nutrients in plants, particularly when associated to the amendments (SB or SB + RP), probably due to several mechanisms. The application of fermented SB (with or without RP) agrowaste also increased soil fertility. Additive and interactive effects of applied treatments (enhancing major nutrients and C sources, symbiotic developments and depressing Cd concentration in

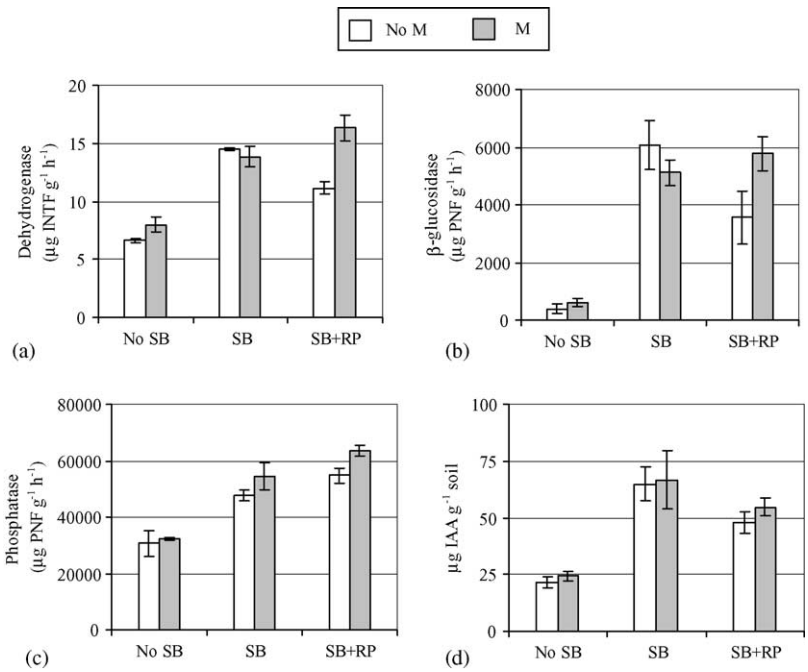

Fig. 5. Dehydrogenase, β-glucosidase, phosphatase activities and indole acetic acid (IAA) production (a–d, respectively) in rhizosphere soil in *Trifolium* plants growing in Cd (5 μg g^{-1})-contaminated soil. Verticals bars represent standard errors.

shoot tissue) significantly enhanced growth and nutrition of *Trifolium* plants growing in Cd-polluted soil.

Measurements in microbial activities in the rhizosphere of this contaminated soil provide a direct information about the functioning of the system. β-Glucosidase is a hydrolytic enzyme involved in the C cycle was totally depressed in non-amended soil, without C substrate (Ceccanti and García, 1994).

The phosphatase activity was also stimulated by AM inoculation; it is surprising that this activity was greater in SB + RP-amended soil than in the rest of treated soils, since soluble P [highest in this amendment as previously determined by Rodríguez et al. (1999)] leads to a decrease of this activity in soil (Azcón and Barea, 1997). Phosphatases are enzymes with relatively broad specificity and capable of hydrolyzing various organic phosphate esters (Alef et al., 1995). The highest activity in AM inoculated and SB + RP-amended soil is in concordance with the highest microbial activity as dehydrogenase values show (García et al., 1997). Dehydrogenase and indole acetic acid accumulation in rhizosphere soil is considered a marker of soil activity. The application of SB amendments here used as substrates was able to promote most of the enzyme synthesis (García et al., 2000) and this phytohormone.

Mycorrhizal colonization did not affect IAA production but it was effective on root growth of non-treated and SB-treated plants.

We can conclude that the success of revegetation in Cd-contaminated soils is highly dependent on the mycorrhizal inoculation using Cd-adapted AM endophytes as well as on the application of fermented agrowaste residue as SB (with or without RP). The effect of these combined treatments not only increased plant growth but also allowed an enhancement of biological and biochemical values in the rhizosphere soil, improving soil quality.

Acknowledgements

This research was supported by the Plan Nacional CICyT AGL2003-05619-CO2-02. We thank Astrid Vivas for supplying the AM fungus inoculum and Pilar Fuentetaja Casado for technical assistance. Almudena Medina is grateful to Junta de Andalucía for the grant. We would like to thank “Azucarera de Jaén” (Spain) for providing the sugar beet residue.

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