Significance of treated agrowaste residue and autochthonous inoculates (Arbuscular mycorrhizal fungi and Bacillus cereus) on bacterial community structure and phytoextraction to remediate soils contaminated with heavy metals

Rosario Azcón, Almudena Medina, Antonio Roldán, Borbála Biró, Astrid Vivas

In this study, we analyzed the impact of treatments such as Aspergillus niger-treated sugar beet waste (SB), PO₄³⁻ fertilization and autochthonous inoculants [arbuscular mycorrhizal (AM) fungi and Bacillus cereus], on the bacterial community structure in a soils contaminated with heavy metals as well as, the effectiveness on plant growth (Trifolium repens). The inoculation with AM fungi in SB amended soil, increased plant growth similarly to PO₄³⁻ addition, and both treatments matched in P acquisition but bacterial biodiversity estimated by denaturing gradient gel electrophoresis of amplified 16S rDNA sequences, was more stimulated by the presence of the AM fungus than by PO₄³⁻ fertilization. The SB amendment plus AM inoculation increased the microbial diversity by 233% and also changed (by 215%) the structure of the bacterial community. The microbial inoculants and amendment used favoured plant growth and the phytoextraction process and concomitantly modified bacterial community in the rhizosphere; thus they can be used for remediation. Therefore, the understanding of such microbial ecological aspects is important for phytoremediation and the recovery of contaminated soils.

1. Introduction

Heavy metals (HM) have caused serious environmental problems and may enter the ecosystem through mining, atmosphere deposition and agrochemicals as pesticides, fertilizer and anthropogenic activities (Liu et al., 1997). The metals can be extracted from the contaminated soil, translocated from soil to the plants and concentrated in shoot.

Some studies have investigated the impact of HM contamination in soils using culture methods and community-level physiological profiling (Kelly et al., 1999; Ellis et al., 2001). Traditional culture methods, however, may provide a false impression of the microbial community as many species may not be detected. Biomass and changes in respiration have also been used as indicators of soil pollution (Bååth, 1989; Chander and Brookes, 1991); however, this will not highlight changes in diversity and therefore functionality of the microbial biomass and may not be a useful indicator of the effects of contamination. Bacterial community shifts may be more helpful in assessing the impact of pollution and molecular analysis based on 16S rDNA genes of soil bacteria has opened up opportunities to investigate total bacterial populations in soils. An increasing number of studies makes use of polymerase chain reaction (PCR) based methods like denaturing gradient gel electrophoresis (DGGE) for characterising complex soil bacterial communities. The advantage of DGGE is that it recognises very small differences in the nucleotide sequence, allowing a description of the community structure expressed as band patterns on a gel that can be used for analysis of changes or shifts in populations of the predominant community members (Ovreas and Torsvik, 1998; Yang and Crowley, 2000).

For phytoremediation purpose we need a better understanding of the interactions between plant and soil microorganisms, particularly beneficial microbes as arbuscular mycorrhizal (AM) fungi and plant growth promoting rhizobacteria (PGPR) (Whitfield et al., 2004). AM fungi improve the growth and biomass accumulation of plants and yield mainly through the mobilization of nutrients from the soil (Toro et al., 1998) as well as this symbiosis may affect plant protection in contaminated soils (Vivas et al., 2003a). Many rhizosphere colonizing bacteria typically produce metabolites, such as siderophores, biosurfactants or organic acids that stimulate plant growth (Glick, 1995) and also may reduce metal availability in the medium. Until now mycorrhizal fungi and bacteria have been found in HM contaminated soils which is an
indication on AM fungal and bacterial tolerance. The inoculation of autochthonous AM fungi and PGPR bacteria have shown to be an important role in bioremediation of sterilized soils artificially contaminated by HM (Vivas et al., 2003a). But the impact of the applications of both groups (AM-fungi and rhizobacteria) on the structure bacterial communities in natural soil with high grade of HM contamination has not been studied.

On the other hand, the application of the sugar-beet (SB) agrowaste positively interacts with beneficial microbial groups inoculated and these components can modify the soil’s physicochemical, biological and fertility properties that improve plant establishment and growth in disturbed soils (Medina et al., 2006). Previous results suggest that the coinoculation of selected and adapted beneficial microorganisms (AM fungi plus bacteria) and the applications of agrowaste optimize plants-soil system under adverse environments. It may be an alternate way for remediation practices. However, the impact of these strategies on plant growth, bioremediation and microbial communities in a natural multicontaminated soil has not been yet considered.

The aim of this study was to test the impact of microbial inoculations and/or the application of treated SB agrowaste on plant growth, nutrition and metal extraction from a natural HM contaminated soil. In addition we test how these treatments change the microbial community composition, structure in this HM multicontaminated soil compared to PO\textsubscript{4}\textsuperscript{2-} fertilization.

2. Materials and methods

2.1. Fermentation process

NB2 strain of Aspergillus niger was used in this study. It had previously been selected as producing citric acid on complex organic substrates as olive cake and others (Vassilev et al., 1986).

SB a lignocellulosic material, was ground in an electrical grinder to 1 mm fragments. It was mixed at a concentration of 10% with half of the SB a lignocellulosic material, was ground in an electrical grinder to 1 mm fragments. It was mixed at a concentration of 10% with half of the

2.2. Soil–plant experiment

The experiment consisted of a completely randomized factorial block with: (1) single mycorrhizal inoculation with autochthonous AM inoculums (AM), (2) single bacterial inoculation with autochthonous bacteria isolate from multicontaminated soil (B), (3) dual autochthonous AM inoculums plus bacteria inoculation, (AM + B), (4) PO\textsubscript{4}\textsuperscript{2-} fertilization and (5) untreated control. These treatments were applied to unamended control soil or soil amended with A. niger-treated SB agrowaste residue. Treatments were replicated five times giving a total of 50 pots.

The multi-contaminated test soil used in the greenhouse experiment was described in Table 1. The test soil (Gorguel) was selected from Murcia province (Spain). The soil was air-dried, sieved to less than 2 mm and mixed with quart sand (<1 mm) to reach 1:1 soil/sand ratio (v/v).

Table 1

<table>
<thead>
<tr>
<th>Parameter</th>
<th>AM (mg kg\textsuperscript{-1})</th>
<th>Control (mg kg\textsuperscript{-1})</th>
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<tbody>
<tr>
<td>pH (H\textsubscript{2}O)</td>
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<td>7.7</td>
</tr>
<tr>
<td>EC (S m\textsuperscript{-1})</td>
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<td>0.22</td>
</tr>
<tr>
<td>Available P (g kg\textsuperscript{-1})</td>
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<td>1</td>
</tr>
<tr>
<td>Water soluble C (g kg\textsuperscript{-1})</td>
<td>41</td>
<td>41</td>
</tr>
</tbody>
</table>

2.3. Selection of metal tolerant microbes

The bacterial strain was isolated from the multi-contaminated soil Gorguel following serial dilutions of soil, 1 g of homogenised soil from the rhizosphere zone was suspended in 100 mL of sterile water (dilution 10\textsuperscript{-2}) and this suspension was further diluted to reach dilution 10\textsuperscript{-4}-10\textsuperscript{-7}. The suspension was sown on agar plates (Gryndler et al., 2000). The bacterial strains selected were the most abundant cultivable bacterial strains in such soil. For inoculation, appropriate pots were sprinkled with 1 mL (10\textsuperscript{8} cell mL\textsuperscript{-1}) of the bacterial strain mixture grown in nutrient broth medium for 24–48 h at 28 °C temperature (Vivas et al., 2003c). The bacterial inoculum was applied to appropriate pots on clover seeds.

The autochthonous mycorrhizal inoculum, also coming from the multi-contaminated soil, was a stable consortium of morphologically determined Glomus mosseae species, from which the most abundant G. mosseae strain was selected. It was bulked in an open-pot culture of red clover and consisted of soil, spores, mycelia and infected root fragments. 10 g of inoculum (1% v/v) were added to appropriate pots at sowing time just below the clover seeds. Part of the non-inoculated plants received KH\textsubscript{2}PO\textsubscript{4} (as 100 g kg\textsuperscript{-1}). This rate was previously used to match the effect of AM fungus on plant growth given an appropriate control for the mycorrhizal bacteria.

A suspension (1 mL\textsuperscript{-1}) of the diazotrophic bacterium Rhizobium leguminosarum bv. trifolii (10\textsuperscript{8} cell mL\textsuperscript{-1}) was sprinkled over the seeds of all pots at the time of sowing.

Non-mycorrhizal treatments received the same amount of autoclaved inoculum together with a 2 mL aliquot of a filtrate (<20 μm) of the AM inoculum to provide a general microbial population free of AM propagules.

2.4. Growth conditions

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 was 500 μmol m\textsuperscript{-2} s\textsuperscript{-1} as measured with a light meter (LICOR, model LI-188B). Water loss was replaced daily by top watering (tap water).

2.5. Parameters measured

2.5.1. Biomass production, nutrients and metal concentrations

At harvest (3 months after planting) the root system was separated from the shoot and dry weights were measured after drying in a forced-draught oven at 70 °C for 2 d. Shoot concentrations of K

Table 1

<table>
<thead>
<tr>
<th>Parameter</th>
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</tr>
<tr>
<td>Available P (g kg\textsuperscript{-1})</td>
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</tr>
<tr>
<td>Water soluble C (g kg\textsuperscript{-1})</td>
<td>41</td>
<td>41</td>
</tr>
</tbody>
</table>

1 Values that exceed the permissible limits (Council of the European Community, 1986).

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was determined by flame photometry and P (Olsen and Dean, 1965), as well as of Zn, Fe, Cd, Ni, Co, Mn, Mo, Al, Cr, B and Cu were also determined after wet digestion of the air-dried plant samples with HNO₃ + H₂O₂ by inductively coupled plasma atomic emission spectrometry (Takács et al., 2001).

2.5.2. Symbiotic development

The percentage of mycorrhizal root length infected was estimated by observation of fungal colonization after clearing washed roots in 10% KOH and staining with 0.05% trypan blue in lactophenol (v/v), (Phillips and Hayman, 1970) Quantification was performed using the grid-line intersect method (Giovannetti and Mosse, 1980).

2.5.3. Production of indole-3-acetic acid by the bacteria

The bacterial isolate was cultivated at 28 °C in nutrient broth. The production of indole-3-acetic acid (IAA) by this bacterium was measured by the method of Wöhler (1997). The bacterium was grown in nutrient broth and then collected by centrifugation at 1000g for 5 min. The bacterial pellet was then incubated at 37 °C for 24 h with 3 ml of phosphate buffer (pH 7.5) with glucose (1%) and 2 ml of L-tryptophan (1%). After incubation, 2 ml of 5% trichloroacetic acid and 1 ml of 0.5 M CaCl₂ were added. The solution was filtered through a Whatman filter paper No. 2. Three milliliter of the filtrate was transferred to a test tube and 2 ml of Salper Solution (2 ml 0.5 M Fe Cl₃ and 98 ml 35% per-chloric acid) was added. This mixture was incubated for 30 min at 1000°C for 15 s, 55°C for 30 s; and a final step of 10 min at 72°C. The total reaction mixture of the first PCR consisted of 25 μl with the following ingredients: 1 μl volume (approx 10 ng) of extracted DNA, 1 μm primer P1, 1 μm primer P2, 10 μl Eppendorf Master Mix (2.5X) and sterile Milli-Q water to a final volume. The second amplification was performed by using 1 μl of the products of the first reaction as template. In this, primers P2 and P3 were used under the same conditions described above. PCR products were analyzed by electrophoresis in 2% agarose gels stained with ethidium bromide.

DGGE analyses were conducted using 20 μl of this latter PCR product loaded into a 40–65% urea–formamide–polyacrylamide gel. An INGENYPhorU System (Ingeny International BV, The Netherlands) was run at 75 V for 17 h at 58 °C to separate the fragments. Gels were silver stained with the Bio-Rad Silver Stain according to the standard DNA-staining protocol and photographed under UV light (λ = 254 nm) using an UVitec Gel Documentation system (UVitec Limited, Cambridge, UK). The band patterns were compared in different lanes using the UVImap Analysis software (UVitec Limited, Cambridge, UK). The lanes were normalized to contain the same amount of total signal after background subtraction and the gel images were straightened and aligned to give a densitometric curve. Band positions were converted to Rl values between 0 and 1, and profile similarity was calculated using the Nei and Li's similarity coefficients (Nei and Li, 1979) for the total number of lane patterns from the DGGE gel. The similarity coefficients calculated were then used to construct a dendrogram using the unweighted pair-group method with arithmetical averages (UPGMA).

The species richness on DGGE gels (R) was calculated as the mean number of bands present (Vivas et al., 2008, 2009). The structural diversity of the microbial community was examined by the Shannon index of general diversity H' (Shannon and Weaver, 1963) and Simpson index of dominance D (Simpson, 1949). For these analyses, each band was presumed to represent the ability of that bacterial species to be amplified (Ibekwe and Grieve, 2004; Vivas et al., 2008, 2009). The intensity of the bands was reflected as peak heights in the densitometric curve. The Shannon H' and Simpson D indexes were calculated from the following equations:

\[ H' = - \sum (P_i \log P_i) \]

\[ D = \sum P_i^2 \]

\[ P_i = n_i/N, \quad n_i = \text{height of peak and } N = \text{sum of all peak heights in the curve}. \]

2.6. Statistical analysis

Five replicates were made per treatment and data were subjected to analysis of variance. Differences between means were analyzed by Duncan's multiple range test (P ≤ 0.05). For the percentage values, the data were arcsin square transformed before statistical analysis.

3. Results and discussion

The positive effect of agrowaste as SB and microbial inoculation as tool of bioremediation on the plant growth and the impact on the rhizosphere bacterial community was determined in a natural soil from a multicontaminated area (see soil characteristics in Table 1).

The addition of PO₄⁻ to this soil increased plant development (shoot and root growth) similarly to the dual AM + B inoculation in absence of composted SB (Fig. 1A). In un-amended soil, autochthonous single AM or B inoculation increased the root growth by 76% (AM), by 32% (B) and by 136% the dual AM + B inoculation. Comparing the control plants (without microbial inoculation and
a SB) with AM-inoculated plants differences in shoot biomass ranged from 107% (single AM) to 219% (AM + B). The plants growing in amended soils highly increased shoot and root biomass (Fig. 1a). In the SB applied soil the effectiveness on plant development of single mycorrhizal inoculation was higher than PO$_3^-$ /C$_0^4$ fertilization (Fig. 1a).

Total length of AM colonized roots was highly increased by the amendment. In the natural multi-contaminated soil control plants show 20 cm of root length with AM-colonization but this symbiotic value was slightly depressed by PO$_3^-$ /C$_0^4$ fertilization (Fig. 1b). Microbial inoculations increased this symbiotic development and the highest AM colonization, in soil without composted SB, was obtained in dual AM + B inoculated plants that produced 861 cm of AM colonized roots. In the SB amended soil the mycorrhization was enhanced and the maximum value was observed in single AM inoculated plants (Fig. 1b) that produced 1758 cm of mycorrhal root and similar growth responses than PO$_3^-$ /C$_0^4$ -fertilization having only 228 cm of AM-colonized roots (Fig. 1b). One important effect of SB amendment was the improvement of the mycorrhizal infection as previously showed Medina et al. (2006).

The P and K content in Trifolium plants were widely increased by P-fertilization and particularly by SB amendment. In absence of composted SB the chemical treatment (PO$_3^-$ /C$_0^4$) was the most efficient in increasing these both nutrients in plant shoot. But in presence of composted SB, single AM inoculation was the most effective treatment in increasing the content of these nutrients [by 536% (P) and by 370% (K)] compared to plants growing in non-composted soil (Table 2).

In previous studies we used an artificially contaminated soil applying increasing amounts of single metals as Cd or Zn and the effectiveness on plant development of microbial treatments applied (selected AM fungus and/or bacteria) was a consequence of increasing plant nutrition (N, P and K) and concomitantly a decreasing metals acquisition (Vivas et al., 2003a–c; Medina et al., 2006). The plant growth enhancement by the treatments applied seems to be the result of varied and additional mechanisms as those improving nutrients acquisition and those directly related to metals quelation (Zhang et al., 2005; Wu et al., 2006).

In this study we determined that the IAA production by B. cereus was 6.2 lgm L$^{-1}$. In a previous unpublished study we determined that these bacteria were also able to reduce metals content in the medium when growing in vitro [in medium added of the extract (1 soil/1 H$_2$O v/v) of the multicontaminated test soil]. The metals reduction in the growing medium (as percentage) were

![Fig. 1](https://example.com/fig1.png)

**Table 2**

<table>
<thead>
<tr>
<th>Treatments</th>
<th>P (mg)</th>
<th>K (mg)</th>
<th>Fe (mg)</th>
<th>Zn (mg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>no SB</td>
<td>SB</td>
<td>no SB</td>
<td>SB</td>
<td>no SB</td>
</tr>
<tr>
<td>C</td>
<td>50g</td>
<td>440b</td>
<td>2062g</td>
<td>8263c</td>
</tr>
<tr>
<td>PO$_3^-$</td>
<td>362cb</td>
<td>805a</td>
<td>7049dc</td>
<td>13,094b</td>
</tr>
<tr>
<td>AM</td>
<td>126d</td>
<td>801a</td>
<td>3478f</td>
<td>16,332a</td>
</tr>
<tr>
<td>AM + B</td>
<td>211c</td>
<td>532b</td>
<td>6510dc</td>
<td>13,248b</td>
</tr>
</tbody>
</table>

Values not sharing a letter in common differ significantly ($P \leq 0.05$) from each other.
phytoextraction for most of metals (Fe, Zn, B, Cd, Cr and Ni) particularly associated with AM inoculum (Table 3).

In general, the SB amendment increased the plant’s content of most of the analyzed elements (B, Cu, Mo, Cd, Cr and Mn) but Al decreased or did not change by the SB amendment application (Tables 2 and 3). In non-amended soil, the most effective applied treatments in improving elements content in plants were: single B inoculation for Al, PO$_4$$^{3-}$ fertilizer for B, Cd, Cr, Mo and Ni, being Cu and Mn more accumulated in dual AM + B inoculated plants (Tables 2 and 3). In the case of SB amended soil, plants inoculated with single B increased in the highest extent Al uptake but the highest Mn and Mo contents were determined in PO$_4$$^{3-}$ fertilized plants (Tables 2 and 3). In SB amended soil, single AM inoculated plants matched B, Cd and Ni shoot content with PO$_4$$^{3-}$ fertilized plants and increased value of Cu content (Tables 2 and 3). The dual (AM + B) inoculation was the treatment that more increases Cr content by the plants in SB amended soil (Table 3). These results indicated that phytoextraction was enhanced by the composted SB application (except for Al) and the microbial inocula applied highly improved this effect for whatever metal analyzed.

The HM exert a negative influence on the soil’s microorganisms (Khan, 2005; Birró et al., 2007) which are considered an index to test soil pollution because the HM have a deleterious influence on cell functioning and thus in soil microbial community. Soil quality is related to the rhizosphere microbial groups but few studies have considered the effect of the mycorrhizal and SB amendment on the structure of the bacterial communities and diversity (Maliszewska-Kordybach and Smreczak, 2003; Zhang et al., 2006). Nevertheless, soil application of SB amendment resulted in a practical method because of its advantages in increasing soil fertility in this degraded soil. Metabolizable C compounds from this SB amendment must be applied to the microbes to ensure their growth and activity. However, no information is available about the effect of the soil application of treated agrowastes which include alternative carbohydrate sources on the environmental structure and function of microbial community compared to conventional PO$_4$$^{3-}$ fertilized used in parallel.

In this study, the bacterial-community profiles were generated from DGGE of the amplified soil DNA (Fig. 2a). The denogram analysis by homology (Fig. 2b) and the Shannon’s diversity index (Table 4) show that the SB amendment modified the structure of the bacterial community. We found two groups forming a distinct cluster in a UPGMA dendrogram: One group included the treatments without amendment, that enclosed three subgroups. (a) Untreated control C and PO$_4$$^{3-}$ fertilized treatments, (b) AM treatment, and (c) treatments inoculated with the bacteria B (B and AM + B) (Fig. 2b). Nevertheless, in the presence of SB amendment, the control and microbial inoculation with AM + B formed two very distinct groups, which indicate a significant structural difference between these two treatments (Fig. 2b).

$R$ index indicates the species richness on DGGE gels calculated as the numbers of bands present (Ampe and Miambi, 2000; Vivas et al., 2008). With the exception of the treatment inoculated with B. cereus the SB amendment increased these values in the treatments (Table 4). Treatments inoculated with AM and AM + B plus SB amendment increased by 233% (AM) and by 123% (AM + B) when compared with their respective treatments (AM and AM + B) without amendment. In the non-inoculated (C or PO$_4$$^{3-}$) or B inoculated soils the effect of SB amendment was not (control) or less (PO$_4$$^{3-}$) relevant (Table 4).

As results show bacterial diversity $H$ was positively increased by the treatments applied particularly AM inoculation with SB (Table 4). The Shannon’s diversity index ($H$) is defined as the proportional abundance of species in a community. We found that these values decreased in AM inoculated plants and particularly when both AM and B inocula were applied. These results

### Table 3

<table>
<thead>
<tr>
<th>Treatments</th>
<th>Al (mg)</th>
<th>Cd (mg)</th>
<th>Cr (mg)</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>no SB</td>
<td>SB</td>
<td>no SB</td>
</tr>
<tr>
<td>C</td>
<td>69c</td>
<td>14f</td>
<td>0.2f</td>
</tr>
<tr>
<td>PO$_4$$^{3-}$</td>
<td>79c</td>
<td>37d</td>
<td>0.4c</td>
</tr>
<tr>
<td>M</td>
<td>78c</td>
<td>65c</td>
<td>0.3d</td>
</tr>
<tr>
<td>B</td>
<td>164a</td>
<td>131a</td>
<td>0.2f</td>
</tr>
<tr>
<td>AM + B</td>
<td>103b</td>
<td>36c</td>
<td>0.4c</td>
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</tbody>
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<table>
<thead>
<tr>
<th>Mn (μg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>C</td>
</tr>
<tr>
<td>PO$_4$$^{3-}$</td>
</tr>
<tr>
<td>AM</td>
</tr>
<tr>
<td>B</td>
</tr>
<tr>
<td>AM + B</td>
</tr>
</tbody>
</table>

Values not sharing a letter in common differ significantly ($P < 0.05$) from each other.
would indicate a decrease or even disappearance of some species in the community when massive introduction of others microorganisms (AM and B) are done. Curiously, microbial inoculations (AM, B or AM + B) decreased $H'_0$ diversity index in non-SB amended soil. The negative effect of massive incorporation (inoculation) of particular microbial groups on the structure of the bacterial community is not surprising in our opinion (Table 4 and Fig. 2a). The positive effect of SB amendment (possibly as an extra C source) on such value compensated the decrease in the structure caused by these microbial treatments and reached the highest bacterial structure values in presence of biological treatments applied.
Biodiversity index (H) as well as dominance concentration index (D) significantly varied with the presence of SB amendment (Table 4). The inoculation of AM caused the highest bacterial diversity (4.3) in the presence of the SB amendment. The microbial inoculation of AM or B and particularly AM + B did not affect the structure of the bacterial community in non-amended soil. Although, in SB amended soils such biological treatments greatly increased (H) value by 215% (AM), 163% (B) and 218% (AM + B), when compared with these treatments in non-amended soil (Table 4). The Simpson index of dominance (D) was affected only in non-inoculated control SB-amended soil, where it increased 100% the “D” (0.04–0.08) value (Table 4). This indicates the presence of the predominant bacterial group.

This study evidences that SB amendment is a suitable tool for increasing and changing the bacterial community in rhizosphere of the multicontaminated soil used. Microbial properties of this multicontaminated soil such as biodiversity and dominance index increased by the application of the treated SB agrowaste and concomitantly favoured the plant development. An interesting result is that PO\textsubscript{4}\textsuperscript{3−} fertilization and single AM inoculation (used in parallel) similarly promoted plant biomass but only AM inoculation increased microbial diversity in the presence of SB amendment.

Singh et al. (2008) suggest that AM fungi affect the bacterial community associated with the roots of the plants where high numbers of bacteria are associated with the extraradical mycelium of colonizing AM fungi, but their functions and in situ activities are largely unknown and most have never been characterized. Arthursson et al. (2005) studied the impact of G. mosseae inoculation on the active bacterial communities (in soil by using a molecular approach) and they found that the dominant bacterial species were activated as a result of G. mosseae inoculation but mostly were uncultured bacterial and Paenibacillus species.

The highest metals accumulation in AM inoculated plants is highly interesting since AM inoculation may be used as a phytoremediation practice in most of natural multi-contaminated soils (Gohre and Paszkowski, 2006; Hildebrandt et al., 2007). The microbial treatments applied and SB amendment used also improved physiological plant status and these plant factors may enhance plant HM tolerance which has relevance in phytoremediation.

Ours results are indicative of the positive effect of the amendments and beneficial microorganisms as AM fungi and bacteria, on the microbial soil status and the relevance of such biotechnological management on bioremediation in multi-contaminated soils. In futures studies we are interested in understanding the main microbial activities and mechanisms involved in the positive effects found in this study. Mechanisms that govern the interaction and selection of bacterial species in the multicontaminated soil may be considered in future proposals for bioremediation strategies.

Acknowledgements

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References


Table 4

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<tr>
<th>Treatments</th>
<th>No SB</th>
<th>R</th>
<th>H'</th>
<th>D</th>
<th>SB</th>
<th>R</th>
<th>H'</th>
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<tbody>
<tr>
<td>C</td>
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<td>2.5</td>
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<tr>
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<td>0.03</td>
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<td></td>
</tr>
</tbody>
</table>


