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Effect of a low rank coal inoculated with coal solubilizing bacteria for the rehabilitation of a saline-sodic soil in field conditions

Efecto de un carbón de bajo rango inoculado con bacterias solubilizadoras de carbón para la rehabilitación de un suelo salino-sódico en condiciones de campo

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Juan Guillermo Cubillos-Hinojosa[1]*, Nelson Valero[1] and Arnaldo de Jesús Peralta Castilla[1]

ABSTRACT

The aim of this research was to evaluate changes to several chemical, biological and physical properties of a Salidic Calciustolls, in response to enhancement by treatment with low rank coal (LRC) and coal solubilizing bacteria (CSB) -*Bacillus mycoides, Microbacterium* sp and *Acinetobacter baumannii*- that release humified organic matter (HOM) through biotransformation of the coal. Under field conditions, 5 m^2 plots were treated with the addition of LRC at a dose of 5 kg m^2 and an inoculum of CSB in a suspension of $1x10^8$ bacteria mL^{-1} at a dose of 100 mL m^{-2}. Soil respiration, microbiological activity, lignin peroxidase (LiP), manganese peroxidase (MnP) and laccase (Lac) enzyme activities were quantified. The variables associated with saline-sodic soils - pH, electrical conductivity (EC), sodium adsorption ratio (SAR), exchangeable sodium percentage (ESP), cation exchange capacity (CEC) were measured every two months bulk density (BD) was determined sixth months after the start of the experiment. The LRC application contributed to the decrease of EC, SAR and ESP, but pH levels did not change significantly. Additionally, no significant changes were found in the BD, however the treatment increased the respiration and microbiological activity of soil, stimulated LiP, MnP and Lac enzyme activity, and increased soil CEC. These results suggest the possibility of using the LRC as an HOM source for the rehabilitation of degraded saline soils - a common problem in soils of the Cesar River Valley (Colombia) and in the dry lands of the Colombian Caribbean influenced by open-pit coal mining.

Keywords:
Lignite
Humic substances
Biotransformation of coal
Soil salinity

RESUMEN

El objetivo de esta investigación fue evaluar cambios en algunas propiedades químicas, biológicas y físicas, en respuesta mejorada por el tratamiento con carbón de bajo rango (CBR) tipo lignito y bacterias solubilizadoras de carbón (BSC) -*Bacillus mycoides*, *Microbacterium* sp y *Acinetobacter baumannii*- que liberan materia orgánica humificada (MOH) mediante la biotransformación de este carbón. En condiciones de campo, se trataron parcelas de 5 m^2 con la adición de CBR a una dosis de 5 kg de CBR m^{-2} y un inóculo de las BSC en una suspensión de $1x10^8$ bacterias mL^{-1} en una dosis de 100 mL m^{-2}. Se determinaron la respiración del suelo, la actividad microbiológica, la actividad de las enzimas lignino peroxidasa (LiP), manganeso peroxidasa (MnP) y lacasas (Lac). Las variables asociadas a la salinidad sódica del suelo: pH, la conductividad eléctrica (CE), la razón de absorción de sodio (RAS), el porcentaje de sodio intercambiables (PSI), la capacidad de intercambio catiónico (CIC) se midieron cada dos meses, mientras que la densidad aparente (Da) se determinó seis meses después de haber iniciado el experimento. La aplicación de CBR contribuyó a la disminución de la CE, RAS y PSI, pero los niveles de pH no presentaron cambios significativos. Adicionalmente, no se evidenciaron cambios significativos en la Da, sin embargo el tratamiento logró incrementar la respiración y la actividad microbiológica del suelo, estimuló la actividad de las enzimas LiP, MnP y Lac, y aumento la CIC del suelo. Estos resultados sugieren la posibilidad de utilizar el CBR como fuente de MOH para la rehabilitación de suelos salinos degradados, un problema común en los suelos del Valle del Río Cesar (Colombia) y en las tierras secas del caribe colombiano influenciadas por la minería del carbón a cielo abierto.

Palabras clave:
Lignito
Sustancias húmicas
Biotransformación del carbón
Salinidad de suelos

[1] Departamento de Microbiología. Universidad Popular del César. Dg. 21 No. 29 92, Valledupar, Colombia.
* Corresponding author: <juancubillos@unicesar.edu.co>

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Currently, soil salinization is an environmental factor of worldwide concern, it has been estimated that 20% of total cultivated and 33% of irrigated soils are affected by high salinity (Shrivastava, 2015). There are around 800 million hectares on the planet affected by salts; of these, 397 million are due to salinity problems and 434 million are affected by conditions associated with sodicity (Munns, 2005; FAO, 2005). The high salt concentrations affect plant growth and crop production by limiting their ability to uptake water and nutrients (Abdul-Qados, 2011), and also contribute to soil degradation processes by increasing the dispersion of aggregates, compaction and soil erosion.

The main source of salt in soils comes from the weathering and erosion of rocks and primary minerals formed *in situ* or transported by water or wind. The main causes that generate salinization processes are: irrigation with saline water, groundwater level, evapotranspiration, water percolation through salt materials and seawater intrusion (Metternich, 2003). However, the dynamics of salts are so high that they cannot always be directly associated with the materials that precede the soils, but must take into account climatic, topographical, hydrological and anthropic factors (Gómez, 2004).

In sodic and saline-sodic soils, gypsum ($CaSO_4 2H_2O$) is the most commonly used amendment to maintain soil electrolyte levels that improve its physical and hydraulic properties (Wong *et al*., 2009). The combined application of gypsum-sulfur-compost has also been used, but in most cases has been decreasing due to the high costs of sulfur to farmers. Also the use of new technologies such as biofertilizers (beneficial bacteria, fungi and mycorrhiza), biopolymers and electromagnetism that stimulate microbial activity considerably, improve the soils physical properties affected by salinity due a reduction in compaction and improvements in long-term soil structure (Zúniga *et al*., 2011).

The addition of humidified organic matter (HOM) to soil has been frequently used to contribute to the rehabilitation of degraded lands (Ros *et al*., 2003), due to various studies that have demonstrated the positive effect of the HOM on soil properties (Khaled and Fawy, 2011). This has allowed HOM be recognized as fundamental to the performance of fertilization, crop productivity, soil degradation and erosion reduction, as well as the mitigation of soil desertification; because it improves soil structure and aggregation, hydraulic conductivity, promotes high levels of nutrient retention and increases cation exchange capacity (CEC) (Tejada and González, 2007; Sharif, 2002; Hernández, 2000). Several authors have incorporated different wastes and biosolids composted from green residues, vinasse (produced in sugar mills and with California red worms), among others, as amendments to soils affected by sodicity, favorably influencing some physical, chemical and biological properties of the soil , contributing to an increase in carbon immobilized by microorganisms and microbiological activity and plant growth (Duran *et al*., 2000, Gasca *et al*., 2011, Wang *et al*., 2014, Mogollón-Sandoval *et al*., 2015, Gutierrez *et al.,* 2016). Therefore, the application of organic amendments is an alternative for conditioning soil with these characteristics.

The low rank coals (LRC) such as lignite have a soft, friable consistency, opaque appearance, humidity of 30-45 %, high ash content, low fixed carbon content (low energy content) (Word of coal, 2005) and are considered by-products of open pit mining. LRC asunderstood by its low degree of carbonification is a great source of humic substances (HS) (Peña-Méndez, 2005; Gianoulli *et al*., 2009) and also has high contents of elements that stimulate microbial growth and development (Hölker *et al*., 2002; Tao *et al*., 2009), and, through different mechanisms, its macrostructureallows the release of HS (Peña-Méndez, 2005). Consequently, LRC could be used as an organic amendment for the management of degraded soils (Senesi *et al*., 1996; Chassapis and Roulia, 2008). Among the microorganisms that have the ability to solubilize LRC to generate substances with similar characteristics to HS obtained from LRC by chemical extraction (Filip and Kubát, 2001) are bacteria isolated from coal samples; some genera and species of *Escherichia freundii*, *Pseudomonas rathonis*, *Pseudomonas fluorescens*, *Streptomyces setoni*, *Pseudomonas putida*, *Bacillus* sp., *Staphylococcus*, *Rhodococcus* and others (Laborda *et al*., 1997; Machnikowska *et al*., 2002; Pokorný *et al*., 2005; Valero *et al*., 2014; David *et al*., 2017) have been reported.

In a study conducted by Valero *et al*. (2014), three new LRC biotransformers were reported: *Bacillus mycoides*,

Acinetobacter baumannii and *Microbacterium* sp.; these were isolated from environmental samples with coal residues, with the ability to solubilize LRC, producing up to 300 mg L^{-1} of HS in liquid medium. Subsequently Cubillos-Hinojosa *et al.* (2015) conducted a study where the LRC inoculated with coal solubilizing bacteria (CSB) were evaluated under greenhouse conditions as an organic amendment for a saline-sodic soil and it was found that the effect of the addition of LRC on the biological and chemical properties is greater when applied in conjunction with CSB. The application of LRC 1% and CSB in the Salidic Calciustolls soil promoted short-term biological activity, which was reflected in an increase in soil respiration, hydrolytic enzyme activity in fluorescein diacetate (FDA), ligninolytic enzyme [lignin peroxidase (LiP) and lacsases (Lac) activity associated with LRC biotransformation], and increased cation exchange capacity (CEC). The treatments of saline-sodic soil with LRC and CSB also generated short-term favorable changes in the chemical variables associated with sodium salinity in soil, and showed a decrease in electrical conductivity (EC), sodium adsorption ratio (SAR) and exchangeable sodium percentage (ESP).

In order to give continuity to the experiment conducted by Cubillos *et al.* (2015) under greenhouse conditions, an experiment was proposed under field conditions with the objective of evaluating the effect of the application of LRC and CSB on several chemical, biological and physical properties of a Salidic Calciustolls soil as a strategy to exploit the use of LRC as a source of humified organic matter (HOM) and CSB as an accelerating agent in the release of HOM from coal, allowing it to mitigate and/or rehabilitate soils with salinity problems, considered a common problem in the soils of the "Cesar" Department, located in an area under the influence of open-pit coal mining in the dry region of the Colombian Caribbean.

MATERIALS AND METHODS

This research was conducted in soil classified by Cubillos (2014) taxonomically as Salidic Calciustolls, which showed the following diagnostic characteristics: mollic epipedon, calcic horizon, base saturation in the profile> 50%, EC> 4, ESP> 15%. At these conditions the soil shows problems of degradation by salinization. This soil is located in the lower part of the alluvial fan of Cesar River Valley (Colombia), near the largest coal mining activity in Colombia. Geographically it is located at latitude 23°55'66'' N, longitude 73°13'47'' W. The area corresponds to tropical dry forest, according to Holdridge classification with an average temperature of 28.4 °C, an altitude of 169 m, annual rainfall of 970 mm and relative humidity between 56-74%. The climate is warm and very dry (IDEAM, 2014).

Low rank coal (LRC) samples

In this experiment, the same sample of a lignite type of low rank coal (LRC) was collected and used in the study by Cubillos-Hinojosa *et al.* (2015) under greenhouse conditions. It was sieved to obtain particles with a diameter of less than 300 µm, before being added to the soil. The characteristics of this LRC were determined in previous research by Valero *et al.* (2014) and showed a humidity of 28.44%, 11.12% ash, 47.79% volatile substances, a calorific value of 4781 kcal kg^{-1}, 41.09% fixed carbon, and 0.13% S. These characteristics correspond to lignite type LRC due to its high moisture content and volatile materials, and a calorific value lower than 6390 kcal kg^{-1}. The content of C, H, O and N elements in the LRC was 46.04%, 3.26%, 42.95% and 1.38% respectively and the ash minerals were found in values of Fe_2O_3 4.24%, CaO 69.3%, MnO_2 0.14%, MgO 9.37%, SrO 0.89%, K_2O 0.05%, and BaO 0.08%. The content of humic substances (HS) was 45% in extractable NaOH (0.5 N), total humic extract 32.91%, humic acid (HA) carbon 24.31%, and fulvic acid (FA) carbon 8.6%. The risk of toxicity from heavy metals in the LRC applied in the soil was considered low and negligible, because the content of As, Co, Pb, V, Cu, Zn, Ni, Cr, B, Mo and Cd [applying the standard methods of the American Section of the International Association for the Testing of Materials (ASTM)], were found in amounts of 0.71, 2.31, 1.73, 1.66, 0.55, 22.43, 3.35, 2.4, 15.11, 2.52 and 0.08 ppm of each metal, respectively.

Coal solubilizing bacteria (CSB)

In this experiment, the same coal solubilizing bacteria (CSB) evaluated in the greenhouse study by Cubillos-Hinojosa *et al.* (2015) were used. These bacteria (*Bacillus mycoides*, *Acinetobacter baumannii* and *Microbacterium* sp.) had been isolated in a previous study by Valero *et al.* (2012), from the rhizosphere of plants present in the area of accumulation of coal sediments, sediments from the washing of coal and LRC respectively. The CSB were reported by Valero *et al.,* (2011) for the ability to solubilize LRC in solid medium and liquid releasing humified organic matter (HOM).

These bacteria were conserved in the strains bank of the research group in Agricultural and Environmental Microbiology of the Popular University of Cesar (Colombia), and the inoculum of each of the bacteria were reactivated and prepared in a concentration of 1 x 10^8 bacteria mL^{-1} following the methodology used by Cubillos-Hinojosa *et al.* (2015). A pool (mixture of the three CSB) was also prepared at a concentration of 1 x 10^8 bacteria mL^{-1}.

The field conditions trial

The area delimited to develop the experiment was prepared by passing a rigid chisel that would break the compacted soil layers as well as subsequently applied irrigation to generate the same moisture conditions.

The experiment was conducted with randomized complete block design with three repetitions per treatment; the experimental unit consisted of a plot of 5 m^{-2} each separated 2 m from the adjacent plot with a 50 cm high barrier to reduce cross-contamination between treatments by surface flow or wind. In all treatments, the LRC incorporation was performed at a dose of 5 kg m^{-2} and each bacterial inoculum (*B. mycoides, A. baumannii* and *Microbacterium* sp.) separately, and the bacterial pool (mix of three CSB) was applied at the rate of 100 mL m^{-2} in the concentration of 1x10^8 bacteria mL^{-1} (Table 1).

Table 1. Treatments used in the field conditions trial.

Treatments	Name	Description
1	C-BM	LRC + *Bacillus mycoides*
2	C-M	LRC + *Microbacterium* sp
3	C-AB	LRC + *Acinetobacter baumannii*
4	C-P	LRC + Pool
5	C	LRC
6	Control	Absolute control

C coal, LRC low rank coal, BM *Bacillus mycoides*, M *Microbacterium* sp., AB *Acinetobacter baumannii*, Pool (P): mixture of three CSB.

The LRC and CSB were mixed with the topsoil to -20 cm depth and star grass (*Cynodon plectostachium*), adapated to saline-sodic conditions (Más and García-Molinari, 2006), which was previously established in the soil, as a vegetation cover, as well as stimulator of biological activity in the rhizosphere used in all plots.

After two, four and six months of treatments had been applied, soil samples were taken from each plot from 0 to -20 cm depth in order to determine the respiration and microbiological activity of the soil, as well as the activity of lignin peroxidase (LiP), manganese peroxidase (MnP), and laccase (Lac) enzymes (associated with the solubilization of LRC) and the variables associated with sodic salinity: pH, electrical conductivity (EC), sodium adsorption ratio (SAR), exchangeable sodium percentage (ESP), and cation exchange capacity (CEC). Bulk density was determined only six months after treatments were applied.

In each plot soil respiration was determined with the closed incubation technique proposed by Celis *et al.* (2009) with some modifications, - installing an incubation chamber with NaOH (Cubillos-Hinojosa *et al.*, 2015) and after 24 h, the amount of CO_2 released from the samples was calculated (Alef, 1995). The soil microbiological activity was determined by the hydrolysis of fluorescein diacetate (FDA) method proposed by Schnürer and Rosswall, (1982) with some modifications for the soil samples (Adam and Duncan, 2001; Greena *et al.*, 2006). The fluorescein produced by FDA hydrolysis was calculated in mg kg^{-1} of soil per hour (Alvear *et al.*, 2007).

The activity of the enzymes associated with the solubilization of coal LiP and Lac were determined following the protocol used by Cubillos-Hinojosa *et al.* (2015), while the MnP enzyme was determined following the method of Paszcznsky *et al.* (1986), where an enzymatic unit was defined as the Mn amount of enzyme required to oxidize 1 µmol of Mn^{2+} to Mn^{3+} in one minute.

The chemical properties of soil: pH, EC, SAR, ESP and CEC were determined according to the protocols of the soil

laboratory of the Agustin Codazzi Geographic Institute, which are based on Soil Survey Laboratory Methods of the US Department of Agriculture (Burt, 2004) in a saturation paste, and bulk density was performed by the cylinder method.

Statistical analysis

The data were submitted to analysis of variance, significant minimum differences and in some cases, the average Dunnett's test was applied by performing a previous analysis of the of the data normality parameters. Additionally, the data corresponding to the last sampling (sixth month) were submitted to Categorical Principal Components Analysis (CATPCA), using the statistical analysis program SPSS version 18 to determine the association between all variables.

RESULTS AND DISCUSSION

Soil respiration

After two months of soil treatment, an increase in soil respiration was found with significant differences (P<0.05) compared to the control in the C-AB, C-BM and C-P treatments. Additionally, it was also observed that C-M and C treatments showed a tendency to increase soil respiration above the control (Figure 1).

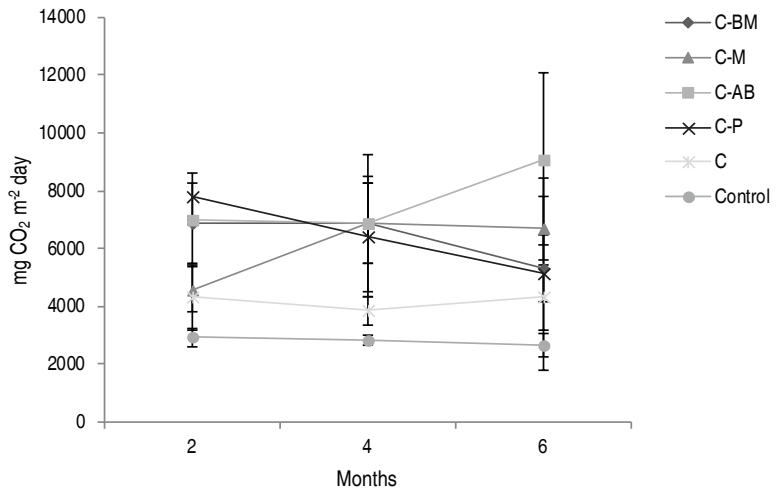

Figure 1. Salidic calciustolls soil respiration after treatment with LRC and CSB in field conditions.
C coal (low rank coal), BM *Bacillus mycoides*, M *Microbacterium* sp, AB *Acinetobacter baumannii*, P mixture of three CSB.

The addition of *B. mycoides, A. baumanii, Microbacterium* sp. and the pool of three BSC in conjunction with LRC stimulated the early activity of soil respiration. This result might indicate the possible microbial activity on the LRC or the stimulation of general soil microbial activity of the soil by LRC influence, due to the possible release of HOM mediated by the CSB that have been reported for their ability to solubilize LRC (Valero *et al*., 2014). The HOM contribute to soil conditioning, aggregate stability of soil (Piccolo and Mbagwu, 1999) and have been used as a coadjuvant in post-mining soil recovery processes (Christanis *et al*., 2006; Valero, 2013; Valero *et al*., 2016).

In the fourth month post-treatment the soil respiration remained constant in C-AB, C-BM treatments, increased in treatment C-M but decreased in C-P. However, all treatments present significant differences with respect to the control. In the sixth month only C-P and C-BM treatments managed to maintain and increase soil respiration, while C-P and C-BM treatments began to decrease in soil respiration but maintained differences with respect to the control. This decrease is probably due to intense microbial activity on the LRC at the beginning with a gradual decrease over time.It is also possible that the native soil microorganism have had some activity on the LRC, as this has been described in long term studies of lignite waste incorporation in the organic matter cycle for the rehabilitation of post mining soil (Rumpel and Kogel-Knabner, 2002).

In addition, these results are consistent with those found by Cubillos-Hinojosa *et al*., 2015 in a previous experiment

under greenhouse conditions, where the addition of LRC 1% in conjunction with CSB increased Salidic Calciustolls soil respiration, showing in field studies (Figure 1) a greater effect when the LRC was applied in conjunction with *A. baumanii* (C-AB treatment), and also showed significant differences with respect to the other treatments and the control. This could occur by the mechanisms and time of solubilization of coal used by the bacteria, indicating a possible effect by the release of HS contributing to the formation of soil aggregates (Piccolo, 2002), which could be influencing the increase in soil respiration.

In the case of treatment C where only LRC was added, there was an increase in soil respiration without the addition of CSB and despite being lower than the other treatments, significant differences from the control were shown. This indicates that LRC, due to its physical properties (high surface area and porosity), stimulates moisture (Levine *et al*., 1982), which favors the microbial growth present in the LRC and the soil, reflected in the increase in soil respiration. These results agree with those reported by Cubillos-Hinojosa *et al.* (2015) when adding LRC 1% in a saline-sodic soil under greenhouse conditions and also by Valero *et al.* (2016) for treating edaphic materials with LRC, where increases in soil respiration without CSB application were observed.

Therefore, the results suggest that the incorporation of LRC as a source of HOM stimulates short-term saline-sodic soil respiration, showing a larger increase when performed in conjunction with CSB, mainly with *A. baumanii*.

Microbiological activity

The results show that after two months of the LRC and CSB application, all treatments showed an increase in the amount of hydrolyzed FDA, which indicate an increase in microbial enzymatic activity in the soil with significant differences ($P<0.05$) compared to the control, presenting a greater increase in the C-P, C-M, C-BM and C-AB treatments respectively (Table 2).

Table 2. Microbiological activity of the Salidic Calciustolls soil post-treatment with LRC and CSB in field conditions.

Treatments	Fluorescein produced (mg kg^{-1} $soil^{-1}$ h^{-1})		
	2 month	4 month	6 month
C-BM	133.8 ± 0.57	130 ± 3.9	113.4 ± 1.7
C-M	141.3 ± 2.3	127.7 ± 2.2	119.4 ± 3.5
C-AB	127.7 ± 4.3	108 ± 7.4	97.9 ± 10
C-P	147.7 ± 2.2	129.7 ± 3.5	127.2 ± 4
C	102.6 ± 3.3	99.8 ± 0.3	92.4 ± 1.3
Control	92.1 ± 6.8	90.9 ± 6.5	91.7 ± 7

C coal (low rank coal), BM *Bacillus mycoides*, M *Microbacterium* sp, AB *Acinetobacter baumannii*, P mixture of three CSB.

The microbiological activity of soil includes all metabolic reactions, cellular interactions and biochemical processes mediated by soil microorganisms (Siqueira *et al*., 1994). The FDA hydrolysis technique allows to measurement of the enzymatic activity of microbial populations, although it is not specific because of sensitivity to the activity of other enzymes such as lipases, esterases and proteases. However, this technique can provide information on estimating changes in microbiological activity (Greena *et al*., 2006), in the saline-sodic soil caused by the addition of LRC and CSB, and because this parameter has been used in several studies as an indicator of soil quality in degraded areas under rehabilitation. In this sense the results suggest that the microbiological activity is stimulated early with the addition of LRC, obtaining a greater increase when LRC is applied in conjunction with CSB. This suggests that the solubilization of LRC by these bacteria result in the release of HS (Valero *et al*., 2014) that can be used by the native soil and are present in the LRC microorganism, favoring microbiological activity. In addition, these results are similar to those obtained in a previous trial under greenhouse conditions, where an increase in microbiological activity was observed in C-P, C-M, C-BM and C-AB treatments (Cubillos *et al*., 2015).

Valero *et al.* (2016) found a stimulus of microbiological activity in the soil in the treatment of edaphic materials (soil materials removed during the pre-mining phase of coal) with LRC and CSB, but its effect was independent of the conjoined addition with CSB.

From the fourth to the sixth post-treatment months a decreasing trend in microbiological activity in all treatments was observed, however all treatments maintained microbiological activity above the control except C treatment. This is possibly due to LRC consumption as a substrate by the microorganisms present as in the same LRC as by the native soil microbiota. These results are similar to those obtained in the previous study under greenhouse conditions by Cubillos-Hinojosa *et al.* (2015).

LiP, MnP and Lac enzyme activity

In Figure 2, an early increase is evident in the activity of lignin peroxidase (LiP) and manganese peroxidase (MnP) enzymes in saline-sodic soil after two months of treatment with LRC and CSB. This is possibly due to the LRC properties, characterized by the presence of element that favor the microbial nutrition and development of CSB (Cubillos-Hinojosa *et al.,* 2015). However, LiP and MnP activity cannot be attributed to the effect of the application of CSB, because peroxidase enzyme activities

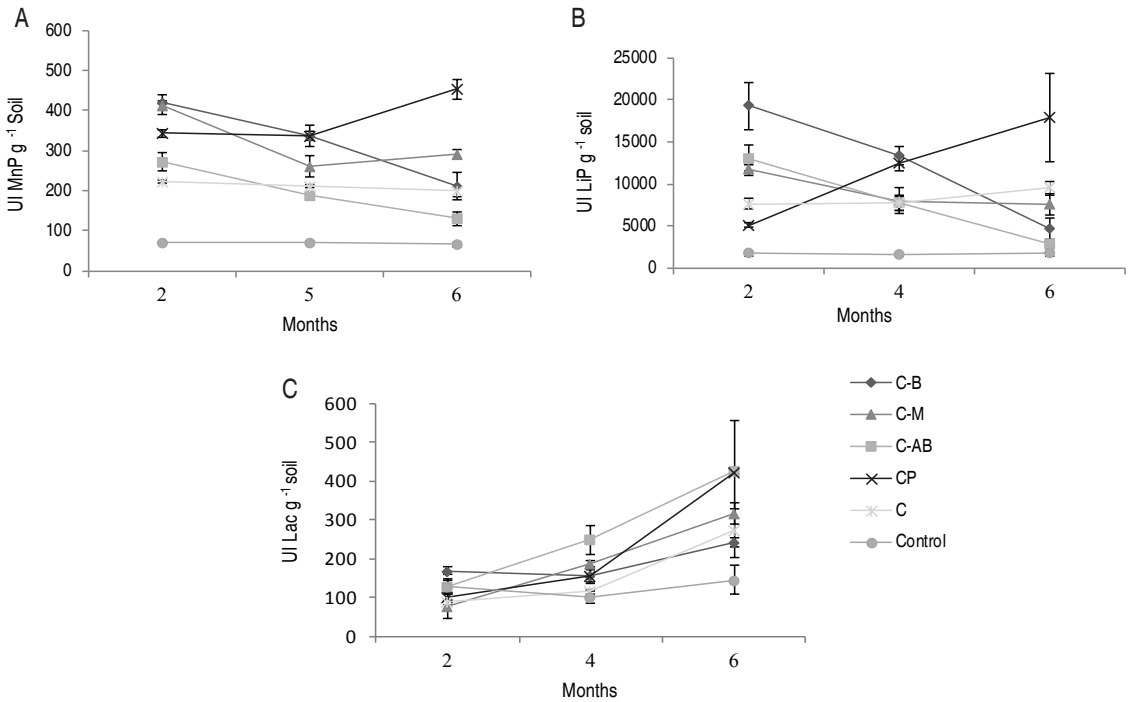

Figure 2. Activity of enzymes associated with coal solubilization in the treatment of Salidic calciustolls soil with LRC and CSB under field conditions. A. Lignin peroxidase (LiP), B. Manganese peroxidase (MnP) and C. Laccases (Lac).
C coal (low rank coal), BM *Bacillus* mycoides, M *Microbacterium* sp., AB *Acinetobacter baumannii*, P mixture of three CSB.

are not known (LiP and MnP) in bacteria. Therefore, this ligninolytic activity may be being performed by fungi found in the microhabitat formed in the porous spaces of the LRC or by native fungi inthe saline-sodic soil. These microorganisms may be being induced to generate LiP and MnP extracellular enzymes that biotransform LRC as described by Fakoussa and Hofrichter (1999).

The activation of ligninolytic enzymes allow fungi to depolymerize the components of the mobile phase of coal, which it is a material of plant origin with a structure very similar to lignin, containing organic matter such as carbon and energy for their metabolism. Also in the process of coal biotransformation some elements important for microbial nutrition such as nitrogen, sulfur and iron can be

available. All these phenomena could have been favoring the colonization and growth of inoculated CSB, the native microorganisms of the LRC, and native microorganism of saline-sodic soil, contributing to increase of microbiological activity as described above.

Figure 2B shows that at 4 and 6 months post-treatment of saline-sodic soil with LRC and CSB, the LiP activity was reduced in most treatments and this is probably due to the fact that some microorganisms can induce MnP activity (Figure 2A) and inhibit LiP activity, this phenomenon of enzymatic regulation has been described in studies of the biotransformation of lignin and coal by Hofrichter and Fritsche (1996).

In all treatments, increases in the activity of Lac enzymes were observed in the fourth and sixth month after soil treatment with LRC and CSB, showing significant differences (P<0.05) with respect to the control treatment. The results suggest that the application of C-AB stimulates Lac activity, showing better results in the fourth and sixth month post-treatment with significant differences (P<0.05) compared to the control (Figure 2C), followed by treatment C-P that showed the greatest long-term increase (6 months post-treatment) in Lac activity. Meanwhile, considering that some bacteria have been reported with Lac activity (Madhavi and Lele, 2009; Diamantidis *et al.*, 2000) and as the CSB inoculated into the saline-sodic soil biotransform LRC, the results show evidence that CSB had Lac activity, especially *A. baumanni*, although it can also be associated with bacteria and fungi native to the LRC used in the experiment or native microorganism in soil.

Chemical variables associated with sodium soil salinity

During the 6 months of the experiment, no significant changes in the pH (P<0.05) were generated according to Dunnett's comparison test (Table 3). After two months post-treatment of saline-sodic soil with the LRC and CSB, the EC of soil decreased in all treatments, showing

Table 3. Chemical variables associated with sodic salinity in Salidic Calciustulls soil post-treatment with LRC and CSB under field conditions.

Treatments	pH			EC (dS m^{-1})		
	Months					
	2	4	6	2	4	6
C-BM	10.0 ± 0.1	9.9 ± 0.0	9.8 ± 0.2	5.3 ± 0.9	5.2 ± 0.7	5.5 ± 0.0
C-M	9.8 ± 0.4	9.8 ± 0.3	9.8 ± 0.1	5.1 ± 0.6	5.1 ± 0.5	5.5 ± 0.8
C-AB	9.5 ± 0.2	9.8 ± 0.2	9.7 ± 0.1	4.3 ± 1.1	4.8 ± 0.9	5.1 ± 0.3
C-P	9.8 ± 0.2	9.8 ± 0.1	9.8 ± 0.1	3.8 ± 0.7	4.4 ± 0.5	5.2 ± 0.7
C	9.6 ± 0.2	9.7 ± 0.1	9.8 ± 0.0	4.2 ± 0.2	4.5 ± 0.0	4.8 ± 0.1
Control	10.0 ± 0.0	10.3 ± 0.11	10.4 ± 0.0	7.9 ± 0.0	7.9 ± 0.0	7.9 ± 0.1
Treatments	SAR (mmol dm^{-3})			ESP (%)		
	Months					
	2	4	6	2	4	6
C-BM	21.6 ± 0.9	21.6 ± 0.3	32.2 ± 0.5	32.3 ± 5.9	37.3 ± 2.8	59.9 ± 1.1
C-M	34.2 ± 1.1	34.2 ± 0.4	35.1 ± 8.4	37.3 ± 0.6	37.3 ± 1.3	55.8 ± 0.7
C-AB	23.0 ± 1.8	23.0 ± 2.9	46.5 ± 3.1	33.6 ± 19.8	33.6 ± 19.5	50.5 ± 18.4
C-P	22.4 ± 8.2	26.3 ± 8.2	44.4 ± 4.6	55.5 ± 3.1	55.5 ± 3.4	58.7 ± 3
C	24.4 ± 3.3	24.4 ± 2.0	31.5 ± 3.2	46.0 ± 13.8	46.0 ± 12.7	59.5 ± 12.5
Control	56.8 ± 0.5	56.8 ± 0.5	56.8 ± 0.3	61.2 ± 1.7	61.2 ± 0.3	61.2 ± 0.7

C coal (low rank coal), BM *Bacillus mycoides*, M *Microbacterium* sp, AB *Acinetobacter baumannii*, P mixture of three CSB, ± SD Standard deviation, EC electrical conductivity, SAR sodium adsorption ratio, ESP exchangeable sodium percentage.

significant differences (P<0.05) in some treatments (C-P, C, C-AB, C-M and C-BM) with respect to the control. In the fourth month, the results showed that only the C-P, C, and C-AB treatments maintained significant differences with respect to the control, and in the sixth month a greater decrease in soil EC was found when treated only with LRC compared to the control by significant differences (P<0.05). Therefore, the addition of LRC alone or in conjunction with the CSB inoculum have an effect on the EC of saline-sodic soil, and agrees with that found by Vance *et al*. (1998), that when applying organic matter with gypsum on the surface of a Natrixeralf soil managed to decrease the EC compared to an untreated control soil.

The SAR and ESP showed significant changes in all treatments by the addition of LRC and CSB in saline-sodic soil. A decrease was observed after the start of the experiment over the 6 months for the SAR, while the ESP showed a decrease in the second and fourth month, with a greater decrease in the C-AB treatment. Also it was evidenced that when the saline-sodic soil is treated with only LRC a greater decrease in the SAR is obtained, while in a study conducted by Gasca *et al.* (2011) where organic matter such as vinasse was applied to treat a soil with salinity problems, it did not show changes in SAR and PSI.

In response to the application of LRC and CSB in the saline-sodic soil the CEC increased in the short-term in all treatments and continuously increased during the experiment showing significant differences (P<0.05) compared to the control. This allows the demonstration of the effect of the LRC as a source of HOM that favorably stimulates the CEC (Table 4) and can be explained because the LRC has a high CEC and its application in the soil favors the growth of native soil microorganism and the application of the CSB can solubilize it and give rise to the release of HS which act as polyelectrolytes that stimulate the CEC (Janos *et al*., 2011).

Table 4. CEC in Salidic Calciustolls soil after being treated with LRC and CSB under field conditions.

Treatments	CEC ($cmol_{(+)}$ kg^{-1})		
	Month		
	2	4	6
C-BM	9.1 ± 1.0	9.2 ± 0.6	9.1 ± 0.5
C-M	9.4 ± 1.3	9.3 ± 0.7	9.1 ± 0.7
C-AB	9.0 ± 1.2	9.0 ± 1.2	9.0 ± 0.8
C-P	9.4 ± 0.3	9.3 ± 0.3	9.1 ± 0.5
C	9.5 ± 0.4	9.2 ± 0.4	9.1 ± 0.3
Control	7.4 ± 1.1	7.4 ± 0.0	7.4 ± 0.4

C coal (low rank coal), BM *Bacillus mycoides*, M *Microbacterium* sp., AB *Acinetobacter baumannii*, P mixture of three CSB, CEC cation exchange capacity.

Bulk density (BD)

At the sixth month post-treatment mark of saline-sodic soil with LRC and CSB, no changes in any of the treatments compared to the control (P<0.05) were observed, where the BD was 1.62 g cm^{-3}. These results are similar to those obtained by Valero (2013) when treating a soil at the start of post-mining rehabilitation in field conditions with LRC and BSC, where no significant differences regarding the control were found. Therefore, these results suggest that the effect that LRC and BSC could have on BD should be considered in the long term.

Categorical principal components analysis

Figure 3 shows the categorical principal components analysis (CATPCA) that was performed to establish the association between the evaluated variables in the sixth month post-treatment of saline-sodic soil with LRC and CSB. The CATPCA explained 57% of the variability of the data in two dimensions: 39% in dimension 1 and 18% in dimension 2 (Table 5). The most influential variables in dimension 1 were LRC, activity of the enzymes LiP, MNP and Lac, soil respiration, pH, EC, CEC and BD of soil, while in dimension 2 it was the Pool of the three CSB and ESP (Table 6).

Table 5. Summary table of the model in CATPCA.

Dimension	Cronbach's Alpha	Variance accounted for	
		Total (Eigenvalue)	% of Variance
1	0.896	6.262	39.138
2	0.685	2.801	17.508
Total	0.949 (a)	9.063	56.646

Table 6. Saturation in components in CATPCA.

Variables	Dimension	
	1	2
C	-0.821	-0.160
C-BM	0.061	-0.067
C-M	-0.036	-0.503
C-AB	-0.518	-0.456
C-P	-0.467	0.832
Microbiological activity	-0.434	0.310
LiP	-0.700	0.567
MnP	-0.797	0.529
Lac	-0.666	-0.026
Respiration	-0.636	-0.451
pH	0.865	-0.215
EC	0.701	0.206
SAR	0.599	0.088
ESP	0.276	0.667
CEC	-0.685	-0.306
BD	0.890	0.284

C coal (low rank coal); BM *Bacillus mycoides*; M *Microbacterium* sp.; AB *Acinetobacter baumannii*; P mixture of three CSB; EC electrical conductivity; SAR sodium adsorption ratio; ESP exchangeable sodium percentage; CEC cation exchange capacity; BD bulk density.

In Figure 3. an association between the following groups of variables was observed: (1) The activity of the enzymes LiP and MnP, microbiological activity and the pool of the three CSB, indicating that the application of the pool of CSB favors the joint expression of peroxidase ligninolytic enzymes and is reflected in the increase in microbiological activity as described above. This is possible because the CSB can biotransform the LRC resulting in the release of HS that might stimulate the activity of native soil microorganisms or LRC companion microorganisms. (2) The activity of the enzymes Lac, LRC, CEC, soil respiration and *A. baumannii* inoculation- This association demonstrates that the LRC application in conjunction with *A. baumannii* favors soil respiration and production of Lac enzymes, which are

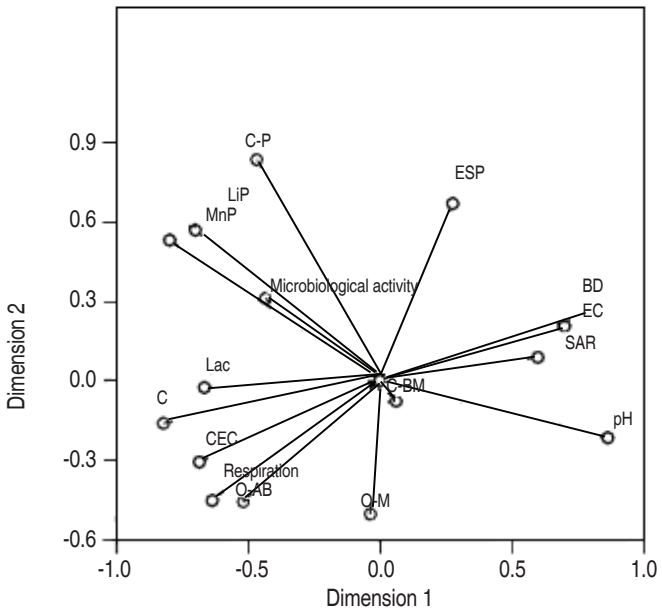

Figure 3. Association analysis of the variables evaluated in a Salidic Calciustolls soil after sixth months post-treatment with LRC and CSB in field conditions.
C coal (low rank coal); BM *Bacillus mycoides*; M *Microbacterium* sp; AB *Acinetobacter baumannii*; P mixture of three CSB; EC electrical conductivity; SAR sodium adsorption ratio; ESP exchangeable sodium percentage; CEC cation exchange capacity; BD bulk density.

generated by bacteria and it is possible that this activity is being performed by *A. baumanni* because it has the ability to biotransform LRC. The LRC favors the CEC, which in itself has a high CEC, due to the presence of phenolic and carboxylic groups (Janos *et al*., 2011). (3) The correlation between BD and the salinity parameters EC, SAR, and pH, indicating that the salinity negatively influenced BD.

CONCLUSIONS

The incorporation of LRC and CSB in the Salidic Calciustolls soil in field conditions favorably influences some chemical and biological properties. This is reflected in a decrease in EC, SAR and ESP, and in an increase of microbiological activity, soil respiration and CEC. The activity of ligninolytic peroxidase enzymes of the native microorganism present in LRC and soil are also favored.

The application of LRC as a source of HOM and CSB in the Salidic Calciustolls soil generates a positive effect on chemical and biological soil properties in the short-term when LRC is added in conjunction with CSB, mainly with *A. baumannii*.

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