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Tolerance and accumulation of lead by endophytic association between *Festuca arundinacea* and *Lewia* sp.

Tolerancia y acumulación de plomo por la asociación endófita entre *Festuca arundinacea* y *Lewia* sp.

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Abstract

Endophyte assisted phytoremediation is an environmentally friendly alternative for the remediation and treatment of soils contaminated with heavy metals. The effect of the association between endophytic fungus (*Lewia* sp.) and *Festuca arundinacea* on the tolerance and accumulation of lead (Pb) was studied after 45 days of culture in contaminated soil. The tolerance to 2,500 mg Pb kg^{-1} was quantified by biomass production and the accumulation of lead in shoots and roots. The phytoremediation potential was quantified by estimating the tolerance index (TI), the bioaccumulation factor (BCF) and translocation factor (TF). The Pb accumulation in roots (14 ± 4.2 mg g^{-1}), was 3-fold higher in plants inoculated in comparison with only plant (3.73 ± 0.64 mg g^{-1}); The TI was 0.76 and 0.56, the BCF was 0.31 and 0.23 for inoculated and non-inoculated plants, respectively. The TF was not detected in both cases, indicates that when working with this association there is not risk of trophic transfer of lead, according to parameters obtained, *Lewia* sp. improved growth and tolerance of *F. arundinacea* in Pb contaminated soil. The association between *F. arundinacea-Lewia* sp. can be considered a promising endophytic association for the remediation *in situ*.

Keywords: Tolerance, bioacumulation, *Festuca arundinacea*, *Lewia* sp., endophyte, lead.

Resumen

La fitorremediación asistida por endófitos es una alternativa ecológica para la restauración de suelos contaminados con metales pesados. Se estudió el efecto de la asociación entre un hongo endofíto (*Lewia* sp.) y *Festuca arundinacea* en la tolerancia y acumulación de plomo (Pb) después de 45 días de cultivo en suelo contaminado. La tolerancia a 2,500 mg Pb kg^{-1} se cuantificó mediante la producción de biomasa y acumulación de plomo en planta. El potencial de fitorremediación se cuantificó estimando el índice de tolerancia (TI), el factor de bioacumulación (FBC) y el factor de translocación (TF). La acumulación de Pb en raíces (14 ± 4.2 mg g^{-1}) fue tres veces mayor en plantas inoculadas en comparación con plantas no inoculadas (3.73 ± 0.64 mg g^{-1}); el TI fue de 0.76 y 0.56, el FBC fue de 0.31 y 0.23 para plantas inoculadas y no inoculadas, respectivamente. El TF fue cero en ambos casos, indicando que cuando se trabaja con esta asociación no existe riesgo de transferencia del Pb a la cadena trófica. *Lewia* sp., favorecio el crecimiento y la tolerancia de *F. arundinacea* creciendo en suelo contaminado por Pb, entonces puede considerarse como una asociación endofítica prometedora para la remediación *in situ*.

Palabras clave: Tolerancia, bioacumulación, *Festuca arundinacea*, *Lewia* sp., endófito, plomo.

1 Introduction

For some years now, heavy metal pollution has become a major environmental and public health problem in Mexico, due to their toxicity (Akter *et al*., 2019). The lead (Pb) is the most common contaminant found in major mining areas, its high half-life makes it a persistent pollutant in ecosystems (Salas-Luévano *et al*., 2017). The presence of heavy metals in soil at levels exceeding the permissible standards has a direct impact on its physicochemical parameters, which limits its productivity and functioning (Wasilkowski *et al*., 2019). By decreasing metal mobility, these processes prevent leaching and groundwater pollution

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and minimize soil erosion and migration of sediments (Ozyigit and Dogan, 2015).

The soil is the common source of biological trace elements that reach man through plants (Salas-Luevano and Vega-Carrillo, 2016). The food chain contamination by heavy metals has become a serious issue because of their potential accumulation in biosystems through contaminated soil, air and water. Pb in plants can be phytotoxic in high concentrations, inhibits energy processes of breathing and photosynthesis causing withering and growth reduction (Kabata-Pendias, 2011). However, there are plants that can tolerate this type of contaminants. An interesting technique environmentally friendly, based on the use of the ability of certain plants to accumulate and/or stabilize heavy metals on soil, is the phytostabilization. Many studies have explored the phytoremediation potential of *Festuca arundinacea* on both organic (Cheema *et al*., 2009; Cruz-Hernández *et al*., 2013; Mendarte-Alquisira *et al*., 2016) and inorganic contaminants (Begonia *et al*., 2005; Zhao, *et al*., 2013; Wasilkowski *et al*., 2019). In recent years, there has been a growing interest in studying the role played by microrganisms associated with the roots of these plants. Fungi play vital role in all ecosystems and are capable of regulating the nutrient as well as energy flow through their mycelial networks (Kumar *et al*., 2019). Endophyte fungi, at some stage of their life cycle, are associated with the roots, favoring plant growth and have been shown to have great potential to improve the applicability and efficiency of phytoremediation. Nevertheless, to date, the role of endophyte in phytorremediation studies have not been potentially explored. According to Velázquez-Osornio (2011), the application of this type of fungi to improve metal phytoremediation has been delayed, in part, due to the lack of toxic metal-tolerant strains. The study of such associations and, in particular, the effect of the fungus-plant association in the phytoremediation of soils contaminated by heavy metals is important, represents a tool that can be applied to improve the mechanisms that allow the accumulation of these elements in the stems and/or roots of plants (Sudová *et al*., 2007). The objective of the present study is to evaluate under greenhouse conditions, the effect of endophytic association between *Lewia* sp. (endophyte fungus) and *Festuca arundinacea* (potentially phytoremediation plant) on tolerance, accumulation and stabilization of lead in artificially contaminated soil from the "El Pedernalillo" located in the south of the State of Zacatecas.

2 Materials and methods

2.1 Soil sample

The Pedernalillo is a laggon situated in the southeast of the city of Zacatecas; this site has been the target of different studies over the years due to the contamination of the site, the origins of pollution go back to the virreinal era, consequence of the increasing mining activity. Currently the lagoon is used for agriculture, mainly growing corn and beans, because during a time of year the lagoon dries completely. The soil sample was collected from a depth of 0 to 30 cm on the periphery of the lagoon (22°44'23.2" N, 102°27'30.9" W). It was stored in a dark place at room temperature in plastic containers until later use.

2.2 Physical and chemical parameters of soil

The soil sample was air-dried and passed through a 2 mm sieve to remove stones and roots. Physical and chemical parameters of soil was analyzed: soil texture was determined using the hydrometer method (Bouyoucos, 1962); water holding capacity (WHC) by quantifyin the water contend retainded a saturated soil simple after 48 h (Veihmeyer and Hendrickson, 1931); field capacity by gravimetric method (Ortíz and Ortíz, 1984), cation exchange capacity by ethylenediaminetetraacetic acid titrimetric method (Motsara and Roy, 2008), pH was measured by the potentiometric method, dissolving 1 g in 2.5 mL in water; soil organic matter (SOM) was quantified by Walkley-Black method (Pansu, 2006).

2.3 Artificially spiked soil

The soil sample was artificially contaminated with solution of lead nitrate $Pb(NO_3)_2$ (J.T. Baker, USA), from this solution an aliquot was taken to get a final concentration of 2500 mg Pb kg^{-1}. The contamination was carried out for each experimental unit containing 1 kg of soil previously passed through a 2 mm sieve. The soil was spread over a flat surface forming a layer 2 cm thick, was divided into 30 equal parts. Each quadrant was contaminated and mixed, first, each quadrant separately and after, the quadrants between each other. Subsequently, the soil was dried at room temperature for 48 h. After, the soil samples were taken at three different random points (1 g of dry soil) per experimental unit (n=3), per

three treatments: (i) soil contaminated without plant (control), (ii) soil contaminated and *F. arundinacea* and (iii) soil contaminated and *F. arundinacea-Lewia* sp. The lead concentration was analyzed by flame-atomic absorption spectrometry (AAS).

2.4 Homogeneity of lead in the soil

The homogeneous distribution of lead in the soil was evaluated by Pearson coefficient. Two analyses were carried out for this study: (i) variation within an experimental unit and (ii) variation between experimental units. This coefficient of variation that expresses the change with respect to a reference and was defined according to Eq.1:

$$\text{Pearson coefficient of variation} = \frac{\text{Standard deviation}}{\text{Mean}} \times 100 \quad (1)$$

2.5 Plant material

This study was done with *Festuca arundinacea* Schreb. Plants, obtained from seeds bought at the central supply in Mexico City. A disinfestation was done before sowing, consisted of immersing the seeds in a detergent solution (0.02 g mL^{-1}) for 20 min with constant agitation, then in an ethanol solution (70% v/v) for a 1 min, followed by sodium hypochlorite (10% v/v) for 30 min, finally washed with sterile distilled water. The treated seeds were aseptically sown in culture tubes with 30 mL of sterile Murashige and Skoog medium (Murashige and Skoog 1962) (Sigma-Aldrich, USA) added with 10 g L^{-1} of sucrose (Sigma-Aldrich, USA) and 1.8 g L^{-1} of Phytagel as gelling agent (Sigma-Aldrich, USA), the pH of the medium was adjusted to 5.8, with 1N KOH. Five seeds per tube were grown for 15 d (25 ± 2 °C; 16 h photoperiod light).

2.6 Fungal inoculum and induction of the endophytic association

Lewia sp. was isolated from surface-sterilized seeds of *F. arundinacea* germinated under *in vitro* conditions (Cruz-Hernández *et al*., 2013). The fungus was maintained (4 °C) on potato dextrose agar (PDA) medium after 7 days of growth at 30 °C. For the induction of the endophytic association, the medium used for plant aseptic cultures was also used for fungal culture. The fungal inoculum was prepared by inoculating mycelium discs (5 mm) on Petri dishes containing MS medium with sucrose (10 g L^{-1}) and Phytagel, and incubated at 30 °C for 15 d. Sixty-five discs of peripheral mycelium were cut and the MS medium adhered was removed from each disk with a sterile scalpel. The free-medium biomass disks obtained were aseptically disaggregated by agitation in tubes containing glass beads (3 mm) and isotonic solution (0.9% NaCl) (Mendarte-Alquisira *et al*., 2016). In order to establish endophytic association, 15-day-old plants of *F. arundinacea* were inoculated with 2 mL of the fungal biomass suspension, the solution was injected inside the phytagel around the roots. Inoculated or non-inoculated plants were grown for 15 d more at 25 ± 2 °C (16 h photoperiod). The endophytic nature of *F. arundinacea* and *Lewia* sp. has been confirmed by confocal microscopy; Mendarte-Alquisira *et al*., (2016) observed fungal hyphae compartmentalized into epidermal root cells of *F. arundinacea*.

2.7 Phytoremediation study

After 15 d under *in vitro* conditions and 20 d in model soil for acclimatization, five seedlings (*F. arundinacea*) or five seedlings associated (*F. arundinacea-Lewia* sp) were randomly transplanted to each plastic pot containing spiked or natural soil (1000 g), respectively. Four sets of treatments (n=5) were analysed simultaneously: (i) soil not contaminated and *F. arundinacea*, (ii) soil not contaminated and *F. arundinacea-Lewia* sp.,(iii) soil contaminated and *F. arundinacea*, and (v) soil contaminated and *F. arundinacea-Lewia* sp. Modified Long-Ashton solution was added as a nutrient solution the day of transplant: KNO_3, 808 (g L^{-1}); $Ca(NO_3)_2 4H_2O$, 944 (g L^{-1}); $NaH_2PO_4 \cdot H_2O$, 184 (g L^{-1}); and $MgSO_4 \cdot 7H_2O$, 368 (g L^{-1}). Plants were kept in greenhouse conditions at 23-28 °C with photoperiods of 16 h and were watered every 2 or 3 d, depending on the evaporative demand. After 45 d, the plants were harvested, separated into roots and shoots, the roots were washed with 10 mM of ethylenediaminetetraacetic acid (EDTA) to remove possible residues of extracellular Pb as well as soil particles attached, roots and shoots were dried separately in a hot air oven (60 °C, 48 h) to determine the biomass accumulation. The roots and shoots lengths were measured using a 30 cm graduated clear plastic ruler.

2.8 Analytical methods

2.8.1 Lead analysis in plant tissues

In order to quantify lead accumulated in plant tissue, roots were washed with 10 mM EDTA to remove the adsorbed Pb, Then, the roots and shoots were oven-dried (60 °C) until constant weight. Per each 100 mg of dry tissue was completely digested in a microwave digestion system (CEM, MARSXpress) with 5 mL of HNO_3 (J.T. Baker, Instra-analyzed) and 4 mL of deionized H_2O (18 MΩ cm^{-1}, *Milli-Q Millipore*). Samples were filtered (0.45 μm, *Whatman*) and the final volume was adjusted to 10 mL with deionized water (Rojas-Loria *et al*., 2014).

2.8.2 Extraction of total and soluble lead in the soil

The concentration of total and soluble lead was quantified as indicated in NOM-147- SEMARNAT 2004. Soluble lead: the soil sample (0.5 g) was mixed with 10 mL of H_2O-CO_2 (120 rpm, 24 h). The aqueous phase was separated by centrifugation and it was filtered with nitrocellulose membranes (GN-Metricel, 0.45 μm). Total lead: 0.5 g of soil was completely digested in a microwave digestion system (CEM, MARSXpress) with 10 mL of HNO_3 (J.T. Baker, Instra-analyzed). Samples were filtered (GN-Metricel, 0.45 μm) and the final volume was adjusted to 25 mL with deionized water.

2.8.3 Quantification of heavy metal

The Pb concentration in the extracts was analyzed by AAS using a wavelength of 283 nm with a mixture of acetylene air gas (1.5-2 L min-1) and a burner opening of 0.5 nm (Shimadzu, AA-6300).

2.8.4 Determination of parameters as indices of phytoremediation potential

The tolerance index (TI), translocation factor (TF) and bioconcentration factor (BCF) were used in determining the remediation potential of *F. arundinacea* inoculated and non-inoculated with *Lewia* sp. The TI is calculated as the biomass of a plant grown in the presence of a metal divided by the plan biomass of a control (Diwan *et al*., 2010). The TF is used to work out the ability of plants to translocate heavy metal from roots to harvestable aerial plant parts (Deng *et al*., 2004). Finally, the BCF is defined as the ratio of the metal concentration in plant to the metal concentration in the soil (Diwan *et al*., 2010). These were calculated by the following formulas:

$$TI = \frac{\text{Plant biomass with} Pb}{\text{Plant biomass without } Pb} \quad (2)$$

$$TF = \frac{[Pb^{2+}]shoot}{[Pb^{2+}]root} \quad (3)$$

$$BCF = \frac{[Pb^{2+}]plant}{[Pb^{2+}]soil} \quad (4)$$

2.9 Statistic analysis

Statistical analysis of the data was carried a variance analysis (ANOVA) using the Tukey-Kramer test and the comparison of mean values was done Student's t-test available in the SPSS statistical package ($P < 0.05$). The results are presented as mean values and their associated standard error deviations. In growth variables, each value is the mean of 5 plants. In biochemical parameters, each value corresponds to the mean of 5 replicates (5 plants per replicate).

3 Results and discussion

3.1 Physical and chemical parameters of soil

The adsorption of metals is strongly related by the soil pH and therefore the major factor affecting metal availability for plant uptake, Lead (Pb^{2+}), for example, predominates in soil with a pH <6, and changes to the form $PbOH^+$ (solid phase) at pH levels between six and eleven. (Liphadzi and Kirkham, 2005). The average pH value obtained was 6.40, this value corresponds a slightly acidic soil. Thus, the nutrients can be more easily available to *F. arundinacea*, by contrary, lower bioavailability of Pb. However, the immobilisation of metals in soils is highly complex. It depends on the nature of the organic matter and the other soil components and the chemistry of the metals (Staunton, 2002). The field capacity found was 23.7%, representing the water quantity which a certain, initially saturated soil is still able to hold against gravity after 2-3 days. The original particle size distribution (56.8% sand, 20% silt, and 23.2% clay) allowed us to identify the soil sample as a sandy-clay-crumb soil. The average pore space was 58.4%. According to Reyes (1996) if a soil presents a porous space of 50% is shared equally by both water and air; under these conditions the soil is excellent for cultivation. The organic matter content was 3.14% and

the average cation exchange capacity was 17.3%. The clay mineral and organic matter components of soil have negatively charged sites on their surfaces which adsorb and hold positively charged ions (cations) by electrostatic forces. Thus, a polarisable acid, such as lead, could be associated with organic ligands, allows us to know that a part of this bioavailable cation was in the soil for phytoremediation studies.

3.2 Homogeneity of lead in the soil

The homogeneity of lead in the soil was determined by the coefficient of variation. This analysis was performed to find out the variation within an experimental unit and to find out the homogeneity between experimental units. Analyzing the lead at three different random points within the experimental units (n=3). The variation within an experimental unit was (i) 9.57-18.38% of the soil contaminated without plant (control), (ii) 1.16-24.79% of the soil contaminated and *F. arundinacea* and (iii) 1.67-31.6% of the soil contaminated and *F. arundinacea-Lewia* sp. The variation between experimental units was (i) 12.67% of the soil contaminated without plant (control), (ii) 11.95% of the soil contaminated and *F. arundinacea* and (iii) 10.88% of the soil contaminated and *F. arundinacea-Lewia* sp. Up to 20% of heterogeneity within the experimental unit was proposed as acceptable. The coefficient of variation between experimental units was 10.88-12.67%, assuring homogeneity of the inorganic contaminant at the time of transplantation.

3.3 Plant tolerance and accumulation of lead

As effective metal phytoremediation strategies depend on the ability of the plant to tolerate and accumulate metals from the environment, the wide prevalence of endophytic fungi and their potential to modulate metal speciation, toxicity, and mobility make them a key component of any remediation effort (Yousaf, 2013). The benefits of the endophytic interaction include improved plant growth, higher nutrient content, insect pest and herbivore resistance, resistance or disease tolerance, increased competitiveness enhanced tolerance to stressful factors such as heavy metals, low pH, and high salinity (Uzma et al,. 2019). A first parameter for measuring efficiency is the optimal growth of plants at the contaminated soil compared to growth in uncontaminated soil (Cruz-Hernández *et al*., 2013, Mendarte-Alquisira *et al*., 2016).

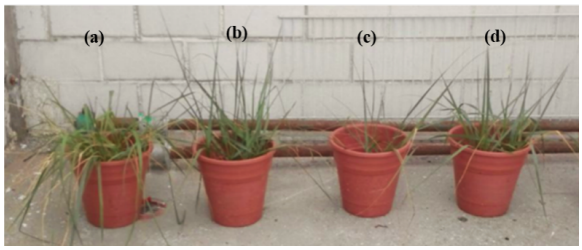

Fig. 1. *F. arundinacea* growing on soil after 45 days of culture in greenhouse conditions at 23-28 °C: (a) contaminated soil and plant inoculated, (b) soil not contaminated and plant inoculated, (c) contaminated soil and plant non-inoculated, (d) soil not contaminated and plant non-inoculated. The initial concentration was 2,500 mg Pb kg^{-1}.

Table 1. Final concentration of lead (Pb) in roots and shoots of *F. arundinacea* (non-inoculated) and endophytic association *F. arundinacea-Lewia* sp. (inoculated) after 45 days of culture on soil.

	Roots		Shoots	
	Non-inoculated	Inoculated	Non-inoculated	Inoculated
Concentration (mg Pb g^{-1} dry tissue)	3.73 ± 0.64^{a}	14 ± 4.2^{b}	n.d	n.d
Concentration (%)	0.25 ± 0.06^{A}	0.73 ± 0.12^{B}	n.d	n.d

Different letters represent significant differences accord to Student's t-test ($P<0.05$). Uppercase (%) and lowercase (mg Pb g^{-1} dry tissue). The initial concentration was 2,500 mg Pb kg^{-1}; n.d. not detected. In growth variables, each value is the mean of 5 plants. In biochemical parameters, each value corresponds to the mean of 5 replicates (5 plants per replicate).

In this study, the association *F. arundinacea-Lewia* sp. was able to tolerate 2,500 mg Pb kg^{-1} without symptoms of phytotoxicity like atrophy, necrosis or pigmentation (Fig. 1). Although, root and shoot length of *F. arundinacea* had not differences significatives after 45 days of growth in contaminated soil with or without inoculation with *Lewia* sp. (Fig. 2), the biomass production was stimulated by the presence of *Lewia* sp. after 45 days of exposure by Pb (Fig. 3). A significant effect on root and shoot biomass production was observed by the presence of *Lewia* sp., similar results have been observed in the association of *F. arundinacea* and Neothypodium coenophialum on soil contaminated with Zn (Zamani *et al*., 2014). Contrary to the observed in non-inculated plants, these had a low biomass production in either roots or shoot, this same effect was observed by Begonia *et al*. (2005) in the growth of *F. arundinacea* cultivated at 1000 and 2000 mg Pb kg^{-1} and EDTA, which may be a consequence of abiotic lead stress. As a result, *Lewia* sp. clearly stimulated biomass production when *F. arundinacea* was grown in contaminated soil; some authors have proposed that endophytes could cause an elongation of the vegetative growth period (Zabalgogeazcoa *et al*., 2006, Lledó *et al*., 2015). The ability to phytoaccumulate lead was determined after 45 days, quantifying the concentration of lead in plant tissue. Table 1 shows lead accumulation in roots of *F. arundinacea* inoculated and non-inoculated with *Lewia* sp. Pb was not detected in shoots, *F. arundinacea* inoculated and non-inoculated with *Lewia* sp. did not have the ability of transferring metal from root to shoot.

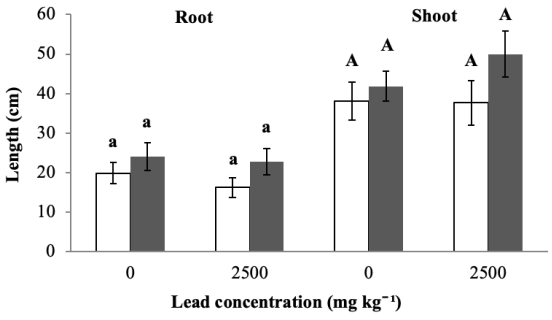

Fig. 2. Shoot and root length of *F. arundinacea* after 45 days of growth in contaminated soil with or without inoculation with *Lewia* sp. Different letters represent significant differences accord to Tukey-Kramer test (P<0.05, n=15). Open columns non-inoculated and filled columns inoculated.

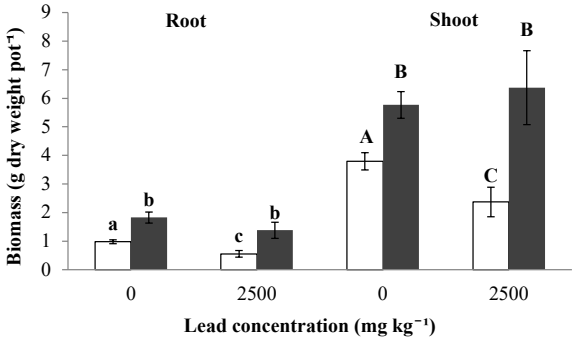

Fig. 3. Biomass production by *F. arundinacea* and *F. arundinacea* associated with *Lewia* sp. after 45 days of growth in contaminated soil. Different letters represent significant differences accord to Tukey-Kramer test (P<0.05, n=15). Open columns non-inoculated and filled columns inoculated.

The non-inoculated roots accumulated 0.25% of the initial concentration of lead by experimental unit. However, Pb accumulated in inoculated roots (14 mg Pb g^{-1} dry tissue) was significantly increased in comparison with roots non-inoculadas (3.73 mg Pb g^{-1} of dry tissue). Other studies have similar results; according to Steliga and Kluk (2020) Pb accumulated in roots by *F. arundinacea* was 1.269 mg g^{-1} dry tissue after 6 months of culture in the soil with diverse concentrations of heavy metals. The general trend from some plant species are that the root tissues accumulate significantly greater concentrations of metals than shoots, it could be affected by several factors: (i) the high metals availability for the plant, (ii) its limited mobility, once inside the plant or (iii) pH and nutrients in the soils/sediments affected metal uptake by plants (Deng *et al*., 2004). Accordingly, our results showed that *Lewia* sp. improved the bioaccumulation of lead in roots of *F. arundinacea*.

3.4 Phytoremediation potential

The capacity of plants with and without *Lewia* sp. to phytoacumulate was estimated by quantifying of phytoremediation potential parameters (Table 2). Another parameter used for measuring the phytoremediation eficiency is the TF, representing the heavy metals transport from root to shoot (Buendía-González *et al*., 2019). The TF values for all cases were less than 1%, i.e. there is no significative translocation, *F. arundinacea* inoculated and non-inoculated had not the ability to translocate heavy metal from roots to shoots.

Table 2. Determination of tolerance index, translocation factor and bioconcentration factor in plants with and without inoculation with *Lewia* sp. after 45 days of culture on soil.

Treatment	Tolerance index	Translocation factor	Bioconcentration factor
Festuca arundinacea	0.56 ± 0.05^{a}	n.d	0.23 ± 0.03^{A}
Festuca arundinacea-Lewia sp.	0.76 ± 0.04^{b}	n.d	0.31 ± 0.06^{A}

Different letters represent significant differences accord to Student's t-test (P<0.05, n=3). Uppercase (tolerance index) and lowercase (bioconcentration factor). The initial concentration was 2,500 mg Pb kg^{-1}; n.d. not detected.

Soleimani *et al*. (2019) analyzed endophyte-free plants, they observed that *F. arundinacea* is a non-transloca Pb species to shoot; lead is one of the most common causes of poisoning in herbivorous animals, with cattle being the most commonly affected species (Suttle, 2010), this evidence suggests that our technological approach in the laboratory could also be applied in the field, due to the absence of translocation of contaminants from roots to outbreaks mean that toxic organic pollutants are not transferred to the food chain (Gao and Ling 2006, Cruz-Hernández *et al*., 2013). The lead removal efficiency obtained by *F. arundinacea* inoculated was 51% after 45 days of culture in the soil. Alcázar-Medina *et al*., (2019) reported 99.8% the lead removal efficiency in aqueous solution by spherical agglomeration using an extract Agave lechuguilla as biosurfactant. The bioavailability of heavy metals in the soil is therefore, of paramount importance for successful phytoextraction. The concentrations of Pb found in roots indicate that both plant and association can not be considered hyperaccumulative. However, endophytic associations increased the accumulation of Pb in roots, it could be to the fungal mycelium binding to more metals and therefore reducing the toxicity of the metal and therefore the accumulation capacity by the plant (Colpaert *et al*., 2011). The TI values were 0.76 y 0.56 for *F. arundinacea* inoculated and non-inoculated, repectively. The TI values were lower than 1 indicates a net decrease in biomass and a stressed condition of plants (Diwan *et al*., 2010). Buendía-González, 2019 used the TI values to characterize the efect of heavy metal stress in root tissue; P. laevigata seedlings exhibited TI values close to 1 (0.85) for the Pb treated seedlings *in vitro* cultures. Therefore, *F. arundinacea* may not be as effective for accumulation of Pb as that reported for other species. Nevertheless, *Lewia* sp. improved the capacity to tolerate and phytostabilize Pb. Phytoremediation indexes suggest that *F. arundinacea-Lewia* sp. have great potential for field use.

Conclusions

To our knowledge, this is the first study that used the endophytic association between *F. arundinacea* and *Lewia* sp. in the treatment of contaminated soil with metal contaminants (lead). *F. arundinacea* can be an efficient plant for use in phytoremediation. The association between *F. arundinacea* and *Lewia* sp. (endophyte fungi) can be considered a promising endophytic association for the remediation *in situ*.

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References

Akter, T., Khan, S. and Rahman, M. (2019). Toxic elements in Bangladesh's drinking water. In: *Environmental Contaminants: Ecological Implications and Management* (Bharagava, R.N., ed.), Pp 273-296. Springer, Singapore.

Alcázar-Medina, F., Núñez-Núñez, C., Rodríguez-Rosales, M., Valle-Cervantes, S., Alarcón-Herrera, M., and Proal-Nájera, J. (2019). Lead removal from aqueous solution by spherical agglomeration using an extract *Agave*

lechuguilla Torr. as biosurfactant. *Revista Mexicana de Ingeniería Química 19*, 71-84.

Begonia, M.T., Begonia, G.B., Ighoavodha, M. and Gilliard, D. (2005). Lead accumulation by tall fescue (*Festuca arundinacea* Schreb.) grown on a lead-contaminated soil. *International Journal of Environmental Research and Public Health 2*, 228-233.

Bouyoucos, G.J. (1962). Hydrometer method improved for making particle size analysis of soils. *Agronomy Journal 54*, 464-465.

Buendía-González, L., Cruz-Sosa, F., Rodríguez-Huezo, M., Barrera-Díaz, C., Hernández-Jaimes, C., and Orozco-Villafuerte, J. (2019). *In vitro* simultaneous accumulation of multiple heavy metals by *Prosopis laevigata* seedlings cultures. *Revista Mexicana de Ingeniería Química 18*, 1167-1177.

Cheema, S.A., Khan, M.I., Tang, X., Zhang, C., Shen, C. and Malik, Z. (2009). Enhancement of phenanthrene and pyrene degradation in rhizosphere of tall fescue (*Festuca arundinacea*). *Journal of Hazardous Materials 166*, 1226-1231.

Colpaert, J.V., Wevers, J.H.L., Krznaric, E. and Adriaensen, K. (2011). How metal-tolerant ecotypes of ectomycorrhizal fungi protect plants from heavy metal pollution. *Annals of Forest Science 68*, 17-24.

Cruz-Hernández, A., Tomasini-Campocosio, A., Pérez-Flores, L.J., Fernández-Perrino, F.J. and Gutiérrez-Rojas, M. (2013). Inoculation of seed-borne fungus in the rhizosphere of *Festuca arundinacea* promotes hydrocarbon removal and pyrene accumulation in roots. *Plant and Soil 362*, 261-270.

Deng, H., Ye, Z.H. and Wong, M.H. (2004). Accumulation of lead, zinc, copper and cadmium by 12 wetland plant species thriving in metal-contaminated sites in China. *Environmental Pollution 132*, 29-40.

Diwan, H., Ahmad, A. and Iqbal, M. (2010). Uptake-related parameters as indices of phytoremediation potential. *Biologia 65*, 1004-1011.

Gao, Y. and Ling, W. (2006). Comparison for plant uptake of phenanthrene and pyrene from soil and water. *Biology and Fertility of Soils 42*,387-394.

Kabata-Pendias, A. (2001). *Trace Elements in Soils and Plants*. CRC Press LLC, New York.

Kumar, A., Chaturvedi, A.K., Yadav, K., Arunkumar, K.P., Malyan, S.K., Raja, P., Kumar, R., Khan, S.A., Kumar, K., Rana, K.L., Kour, D., Yadav, N. and Nath, A. (2019). Fungal phytoremediation of heavy metal-contaminated resources: current scenario and future prospects. In: Recent *Advancement in White Biotechnology Through Fungi. Fungal Biology* (Yadav, A., Singh, S., Mishra, S., Gupta, A., eds.), Pp 437-461. Springer, Cham.

Liphadzi, M.S. and Kirkham, M.B. (2005). Phytoremediation of soil contaminated with heavy metals: a technology for rehabilitation of the environment. *South African Journal of Botany 71*, 24-37.

Lledó, S., Rodrigo, S. and Poblaciones, M.J. (2015). Biomass yield, mineral content, and nutritive value of Poa pratensis as affected by non-clavicipitaceous fungal endophytes. *Mycological Progress 14*, 67.

Mendarte-Alquisira, C., Gutiérrez-Rojas, M., González-Márquez, H. and Volke-Sepúlveda, T. (2017). Improved growth and control of oxidative stress in plants of *Festuca arundinacea* exposed to hydrocarbons by the endophytic fungus *Lewia* sp. *Plant and Soil 411*, 347-358.

Motsara, M.R. and Roy, R.N. (2008). *Guide to Laboratory Establishment for Plant Nutrient Analysis;* FAO. Rome, Italy.

Murashige, T. and Skoog, F. (1962). A revised medium for rapid growth and bioassays with tobacco tissue cultures. *Physiologia Plantarum 15*, 473-497.

Norma Oficial Mexicana NOM-147-SEMARNAT/SSA1-2004. Diario Oficial de la Federación. 2 de marzo de 2007.

Ortíz, V.B. and Ortíz, S.C.A. (1984). *Prácticas de Edafología*. Universidad Autónoma de Chapingo. Chapingo, Mexico.

Ozyigit, I.I. and Dogan, I. (2015). Plant-microbe interactions in phytoremediation. In: *Soil Remediation and Plants: Prospects and Challenges*, (K. Hakeem, M. Sabir, M. Ozturk, A.R. Mermut, eds.), Pp. 255-272. Elsevier, New York.

Pansu, M. (2006). *Handbook of Soil Analysis*. Springer-Verlag, Berlin, Germany.

Reyes, I. (1996). *Fundamentos Teórico-Prácticos de Temas Selectos de la Ciencia del Suelo Parte I.* 53390, Universidad Autónoma Metropolitana Unidad Iztapalapa, Mexico.

Rojas-Loria, C.C., Favela-Torres, E., González-Márquez, H. and Volke-Sepúlveda, T.L. (2014). Role of glutathione and glutathione S-transferase in lead tolerance and bioaccumulation by *Dodonaea viscosa* (L.) Jacq. *Acta Physiologiae Plantarum 36*, 2501-2510.

Salas-Luevano, M.A. and Vega-Carrillo, H.R. (2016). Environmental impact in a rural community due to a lead recycling plant in Zacatecas, Mexico. *Environmental Earth Sciences 75*, 408.

Salas-Luevano, M.A., Mauricio-Castillo, J.A., González-Rivera, M.L., Vega-Carrillo, H.R. and Salas-Muñoz, S. (2017). Accumulation and phytostabilization of As, Pb and Cd in plants growing inside mine tailings reforested in Zacatecas, Mexico. *Environmental Earth Sciences 76*, 806.

Soleimani M., Afyni M., Hajabbasi M.A., Nourbakhsh F., Sabzalian M.R. and Christensen J.H. (2010). Phytoremediation of an aged petroleum contsminated soil using endophyte infected and non-infected grasses. *Chemosphere 81*, 1084-1090.

Staunton, S. (2002). Direct and indirect effects of organic matter on metal immobilisation in soil. *Developments in Soil Science 28A*, 79-97.

Steliga, T. and Kluk, D. (2020). Application of *Festuca arundinacea* in phytoremediation of soil contaminated with Pb, Ni, Cd and petroleum hydrocarbons. *Ecotoxicology and Environmental Safety 194*, 110409.

Sudová, R. and Vosátka M. (2007). Differences in the effects of three arbuscular mycorrhizal fungal strains on P and Pb accumulation by maize plants. *Plant and Soil 296*, 77.

Suttle, N.F. (2010). *Mineral Nutrition of Livestock*. CABI Publishing, United Kingdom.

Uzma, F., Mohan, C.D., Siddaiah, C.N. and Chowdappa, S. (2019). Endophytic fungi: promising source of novel bioactive compounds. In: *Advances in Endophytic Fungal Research, Fungal Biology* (Singh, B.P. ed.) Springer Nature Switzerland.

Veihmeyer, F.J. and Hendrickson, A.H. (1931). The moisture equivalent as a measure of the field capacity of soil. *Soil Science 32*, 181-194.

Velázquez-Osornio, D.T. 2011. *Aislamiento de hongos endófitos de plantas tolerantes a plomo para su potencial uso en fitorremediación*. Tesis de Especialidad en Biotecnología. Universidad Autónoma Metropolitana Unidad Iztapalapa, México.

Wasilkowski, D., Nowak, A., Michalska, J. and Mrozik, A. (2019). Ecological restoration of heavy metal-contaminated soil using Na-bentonite and green compost coupled with the cultivation of the grass *Festuca arundinacea*. *Ecological Engineering 138*, 420-433.

Yousaf, S., Afzal, M., Anees, M., Malik, R.N., and Campisano, A. (2014). Ecology and functional potential of endophytes in bioremediation: a molecular perspective. In: *Advances in Endophytic Research* (Verma, V.C.; Gange, A.C. eds.) Pp 301-320 Springer, India.

Zabalgogeazcoa, I., García-Ciudad, A., Vázquez de Aldana, B.R. and García-Criado, B. (2006). Effects of the infection by the fungal endophyte *Epichloë festucae* in the growth and nutrient content of *Festuca rubra. European Journal of Agronomy 24*, 374-384.

Zamani, N., Sabzalian, M.R., Khoshgoftarmanesh, A. and Afyuni M. (2015). Neotyphodium endophyte changes phytoextraction of zinc in *Festuca arundinacea* and *Lolium perenne. International Journal of Phytoremediation 17*, 456-463.

Zhao, S., Jia, L. and Duo, L. (2013). The use of a biodegradable chelator for enhanced phytoextraction of heavy metals by *Festuca arundinacea* from municipal solid waste compost and associated heavy metal leaching. *Bioresource Technology 129*, 249-255.