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Sewage Water Irrigation and Growth Response of *Leucaena leucocephala* Inoculated With *Glomus intrarradices* and Application of Organic Matter

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Abstract: The aim of this study is to evaluate the growth response of *Leucaena leucocephala* inoculated with *Glomus intrarradices* and application of organic matter and the actual level of contamination with heavy metals Cu, Cr, Zn and Pb in soil irrigated with sewage and clean water. Sewage water is used for irrigation, which creates both opportunities and problems. This is an option to reduce the stress on limited fresh water and help meet the nutrient requirement of crops, but also produces contamination. In the irrigation District 018, Tulancingo, Hidalgo, Mexico, forage for cattle has been irrigated with residual water for several years. To evaluate the level of contamination of two plots, one hectare was irrigated with residual water and another with clean water. In soil the contamination of Cu, Cr and Ni are below the established limits for contaminants. Pb was not found. A comparison of soil irrigated with clean water or sewage water indicated that Cu is nearly twice as concentrated in soil irrigated by contaminated water; Ni is slightly greater; Cr is more abundant. Soil was collected for a greenhouse experiment with *Leucaena leucocephala* (guaje) to observe its growth with inoculation of *Glomus intrarradices* with different amounts of vermicompost. Later a factorial experiment 7×2 completely randomized design with five replications in the greenhouse was established. The variables measured were plant height, stem diameter, root volume, dry weight of biomass and dry weight of roots. The experiment lasted 180 days from planting until harvesting. It is concluded that the inoculation with *Glomus intrarradices* increased the absorption by *Leucaena leucocephala* in nutrient adsorption.

Key words: Legume tree, *Glomus intrarradices*, organic matter, residual water

INTRODUCTION

Reuse of sewage is one of the best options to reduce the stress on limited fresh water available and help to meet the nutrient requirement of crops. Sewage water is either used or disposed of on land for irrigation purposes, which creates both opportunities and problems (Yadav *et al*., 2002; Horswell *et al*., 2003; Kakar *et al*., 2006). The use of raw or untreated sewage can cause accumulation of heavy metals in the soil and phytotoxicity with an impact on the quality of soil and in forage (Gradezi *et al*., 2004; Malla *et al*., 2007). Metabolic activity of soil microorganisms has also been reported to increase when sewage effluent is used for irrigation (Meli *et al*., 2002; Ramirez-Fuentes *et al*., 2002; Gardezi *et al*., 2007).

Leucaena leucocephala is a long-lived tropical legume and is a nutritious forage tree. It has a great variety of other uses: firewood, timber, human food, green manure, shade and erosion control (Gutteridge and Shelton, 1994; Shelton and Brewbaker, 1994). *Leucaena leucocephala* is the mostly widely used species as a valuable fodder shrub for increased animal production in the tropics (Khamseekhiew *et al*., 2001). It is an evergreen forage rich in protein, minerals and B carotene. The plant can also be grazed directly, is well accepted by livestock, particularly goats and is quite resistant to heavy, frequent defoliation (Meissner, 1997). Yields of forage vary with soil fertility, rainfall, altitude, density and cutting frequency from 1-15 t ha^{-1} year. (Shelton *et al*., 1998); the foliage has high nutritive value for ruminant production. With an edible fraction with 55-70% digestibility, 3-4.5% N, 6-10% ash, 30-50% N-free extract, 0.8-1.9% Ca and 0.23-0.27% P (Jones *et al*., 1992).

The capacity of phytoremediation of *Leucaena leucocephala* has been evaluated associated with *Glomus* and *Rhizobium,* concluding that this plant is a good extractor and accumulator of these metals (Habte and Aziz, 1991; Dhalin *et al*., 1997; Gardezi, 2007).

Leucaena leucocephala and arbuscular endomycorrhiza have been used recently for bioremediation in soil contaminated with Cu (Gardezi *et al*., 2004).

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Arbuscular Mycorrhiza Fungi (AMF) give multiple potential benefits to the host plant growing in practically all soils because its roots are colonized by fungi (Smith and Read, 1997). These fungi are used in the biofertilization and as inoculants (Guzmán-Plazola and Ferrera-Cerrato, 1990). These fungi play a central role in nutrient uptake (George *et al*., 1994). The importance of arbuscular symbiosis in crop production and natural ecosystems has been fundamentally linked to its ability to promote mineral nutrition in deficient soils, especially for nutrients with low mobility, such as P, Cr and Cu. As a rule, in nutrient-deficient soils with plants colonized with AMF the uptake of nutrients with low mobility, such as P, is substantially increased.

Gardezi *et al*. (2007) evaluated the response of *Leucaena leucocephala* to inoculation with arbuscular mycorrhiza with different doses of cow manure. They found a positive effect in the variables related to plant growth. They found that the best growth of the plant depends on different treatments of arbuscular mycorrhiza, because the treatments with *Glomus* sp. had higher values for the agronomic variables.

MATERIALS AND METHODS

In the irrigated district 018 in Tulancingo, Hidalgo, Mexico (Fig. 1) forage is produced for cattle in irrigated areas with raw sewage water. The soil is shallow (40 cm) over tepetate (fragipan), and the soil is saturated most of the time by frequent irrigations. Two plots of one hectare each, one with pasture and another without pasture were located. The plot with pasture was irrigated with sewage water for seven years. The other plot was cultivated for corn (*Zea mays*) and irrigated with clean water. For each sub-plot twenty soil samples were taken in a 25×20 m sub-plot at three depths (0-5, 5-10 and 10-40 cm). For the soil analysis the 20 samples were homogenized to obtain 3 samples for each sub-plot. The rest of the soil (about 500 kg) was prepared for a greenhouse experiment.

The study lasted 180 days from transplanting to harvesting and was conducted under greenhouse conditions in the Colegio de Postgraduados, Montecillos, Estado de Mexico in the spring of 2007.

The seeds were sterilized with sodium hypochlorite and pregerminated in plastic trays. The plants were transplanted to polyethylene bags that had been filled with 3 kg of soil.

The inoculation coincided with the transplanting, mixed with 5 g of sand and 1-2 g of alfalfa roots with 80% colonization of *Glomus intrarradices*. In addition 7 levels of vermicompost (V) were applied as a source of organic matter. The vermicompost was prepared with 60 kg of cow manure, 15 kg of residues of melon, and 25 kg of wheat straw, treated for 5 months with the action of earthworms. Two levels of *Glomus* were applied, with and without *Glomus* inoculation; with 7 doses of Organic Matter (OM) as follows: OM 0.0 (0 gV), OM 0.5 (15 gV), OM 1.0 (30 gV), OM 1.5 (45 gV), OM 3.0 (90 gV),OM 6.0 (180 gV) OM 12.0 (360 gV) (Table 1).

The variables evaluated were Plant Height (PH), Stem Diameter (SD), Root Volume (RV) and Dry Weight of Biomass (DWB).

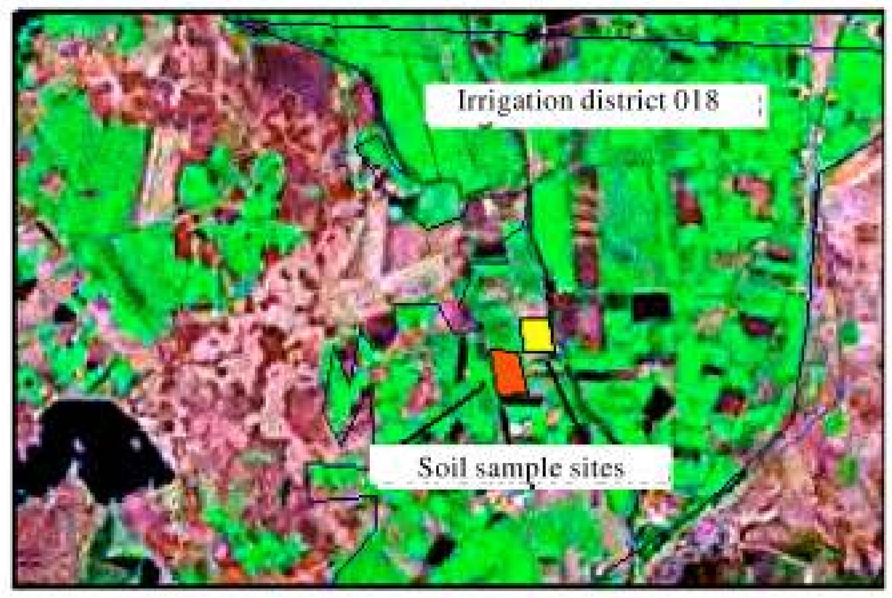

Fig. 1: Localization of soil sample sites in the irrigation district 018, Tulancingo, Hidalgo, Mexico

Table 1: Experimental treatments with vermicompost and *Glomus intrarradices*

Treatments	Organic matter (vermicomposta) Mg ha^{-1}	g/3 kg de suelo	Inoculation with *Glomus*
1	0.0	0	0
2	0.5	15	0
3	1.0	30	0
4	1.5	45	0
5	3.0	90	0
6	6.0	180	0
7	12.0	360	0
8	0.0	0	*Glomus intrarradices*
9	0.5	15	*Glomus intrarradices*
10	1.0	30	*Glomus intrarradices*
11	1.5	45	*Glomus intrarradices*
12	3.0	90	*Glomus intrarradices*
13	6.0	180	*Glomus intrarradices*
14	12.0	360	*Glomus intrarradices*

RESULTS AND DISCUSSION

The soils are shallow (40 cm) underlain by fragipan (tepetate), the soil is irrigated with residual water and saturated with water most of the time. The texture of the soil irrigated with residual water is clay loam in the first five centimeters and clay at 5-40 cm. The soil irrigated with clean water is clay in all the profile.

Table 2 shows the results of the soil sample analysis for each depth for each plot (residual and clean water) and the chemical characteristics of the soil pH, Electrical Conductivity (EC), Organic Matter (OM), Total Nitrogen (TN) and Phosphorus (P) have higher values in the soil irrigated with residual water. The pH is alkaline in both plots and it increases with depth in both soils (except for sample 3). The EC increases from 1.8 to 1.98 dm sec^{-1} from the plot with residual water and increases with depth. This contrast with the plot irrigated with clean water, were the EC is lower than that of residual water, and the EC is lower in samples at more than 0-5 cm. This contrasts also with the plot irrigated with clean water where the EC decreases with depth. The TN is greater in the irrigated soil with residual water at all depths with differences up to 0.09%, with the principal accumulation in the upper horizon for irrigation with residual water. The OM is 4.5% up to 2.87% greater than in clean water, the principal accumulation in clean water is in the upper layer with 2.0%. The horizon of 10-40 cm has lower differences up to 1.3% to those irrigated with clean water. With respect to phosphorus there is a large difference among the two soils, up to 7.75 times greater for the depth of 5-10 cm. The amount of phosphorus is from 140 to 170 mg kg^{-1} and soil irrigated with residual water; in contrast the soil irrigated with clean water had only 20-30 mg kg^{-1}.

With respect to the characteristics of the soil, there is an increase in pH, EC, TN, OM and P (Table 2) which would probably continue to increased with time. The farmer now considers that irrigation with residual water is beneficial because it promotes the high yields of grass. All the metals were more highly concentrated in the upper five centimetres of the soil and decline with depth. However, when in time the increase of organic matter and pH could have toxic effects on grassland due to high pH and inadequate balance of nutrients.

In general there is no contamination with the heavy metals copper (Cu), chromium (Cr), lead (Pb) and nickel (Ni) but their concentration has increased by irrigation with residual water, except for chromium which has a higher level in soil irrigated with clean water (Table 2).

The levels of the soils irrigated with residual water showed differences between residual and clean water. Cu is almost twice the concentration in residual water, Ni is slightly higher, and Cr is less abundant.

Soil analysis shows the presence of Cu, Cr and Ni in low concentrations, and that Pb is absent in the soil.

Table 3 shows the result of analysis of variance: Significant differences were observed among all four variables. When the individual variable was analyzed using factorial analysis, there were significant differences from the principal effects (OM and GI). For the studied variables the behaviour was similar to that found by Gardezi *et al*. (2007).

The plants inoculated with endomycorrhizae fungi (*Glomus intraradices)* were taller than those not inoculated at all levels of organic matter (Fig. 2). However, the curves had differences that are explained by the significant interactions. However, in the inoculated plants there was a positive response to the increase of organic matter up to 6 t ha^{-1}, reducing the highest content in plants not inoculated. The height of the plant was greater

Table 2: Soil analysis for the three depths (0-5, 5-10, 10-40 cm)

Soil sample	pH 1:2	EC (dm sec^{-1})	OM (%) Walkley Black	TN (%)	P (mg kg^{-1})	Cu	Cr	Pb	Ni
						(ppm)			
Soil depth 0-5									
Residual water	8.6	1.80	4.50	0.14	170	0.6896	0.0248	0	0.1014
Soil depth 5-10									
Residual water	8.7	1.90	3.30	0.10	155	0.5103	0.0223	0	0.994
Soil depth 10-40									
Residual water	8.4	1.98	2.20	0.07	140	0.4734	0.0257	0	0.0958
Soil depth 0-5									
Clean water	7.7	0.60	2.00	0.05	30	0.3592	0.0316	0	0.0828
Soil depth 5-10									
Clean water	7.9	0.46	2.00	0.06	20	0.3811	0.0369	0	0.0793
Soil depth 10-40									
Clean water	8.0	0.50	1.63	0.06	20	0.3644	0.0265	0	0.0766

Table 3: Analysis of variance for four variables evaluated in *Leucaena leucocephala*

Source of Variation (SOV)	df	Variables			
		Plant height (cm)	Stem diameter (cm)	Root volume (cm^3)	Dry weight of biomass (g)
Treat	13	792.4978	1.4456	91.8725	64.6989
Organic Matter (OM)	6	857.0619	1.7652	116.9571	82.7476
Glomus intraradices (GI)	1	4888.9286	7.1360	432.5143	248.9143
OM×GI	6	45.19524	0.1775	10.0143	15.9476
Error	56	11.2571	0.0221	2.4214	3.6500
MSD	56	7.4373	0.3293	3.4494	4.2350
CV (%)		5.5694	9.4156	15.8324	16.5924

MSD: Minimum Significant Difference, CV: Coefficient of Variance

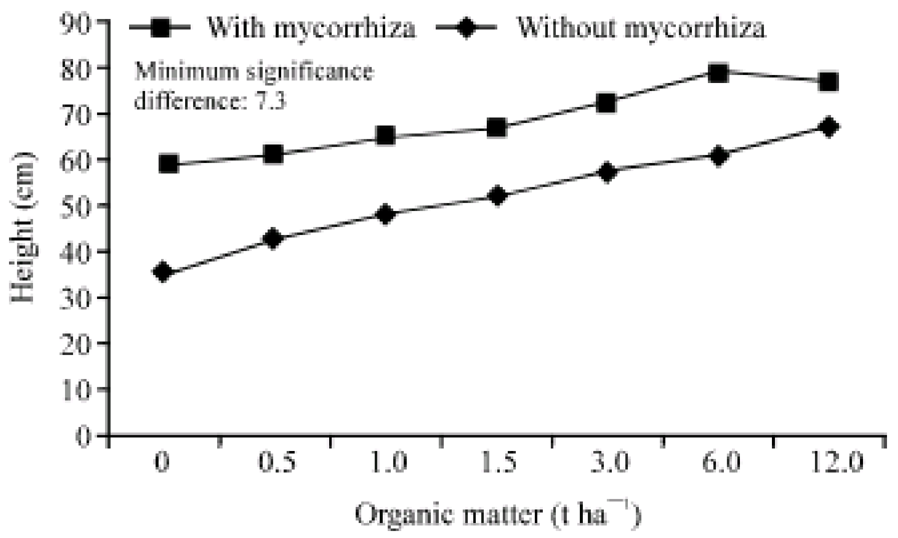

Fig. 2: Effect of arbuscular endomycorrhizae fungi and different levels of organic matter in plant height of *Leucaena leucocephala*

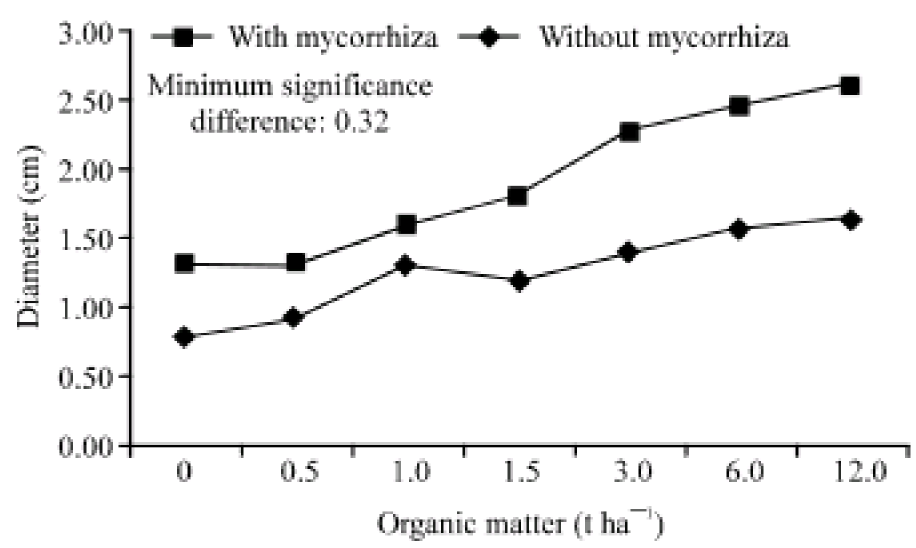

Fig. 3: Effect of arbuscular endomycorrhizae fungi and different levels of organic matter in stem diameter (cm) of *Leucaena leucocephala*

when the content of organic matter increased in the total range studied. Earlier study with *Erythrina americana* Miller, *Sesbania emerus* Aubul, *Dodonea viscosa*, *Dodonea angustifolia* L.f. (Gardezi *et al*., 1995, 1999, 2000) reported that the arbuscular endomycorrhizae fungi stimulate the growth of the plant more than phosphorus fertilization.

There were different tendencies in stem diameter. For plants inoculated with endomycorrhiza (*Glomus intraradices*) there was an increase in stem diameter. With addition of 0 and 0.5 t ha^{-1} there was no increase, but in 1 to 12 t ha^{-1} of organic matter the stem diameter increased progressively (Fig. 3).

The volume of roots of *Leucaena leucocephala* increased in the presence of Mycorrhizae *Glomus intraradices* (Fig. 4). The inoculated and not inoculated plants show a well defined tendency from 0 to 1 t ha^{-1} of OM. The increase of organic material also increases the root volume, especially in inoculated plants up to 6 t ha^{-1}. The root volume was the same at 6 and 12 t ha^{-1}. However, the root volume without inoculation decreased.

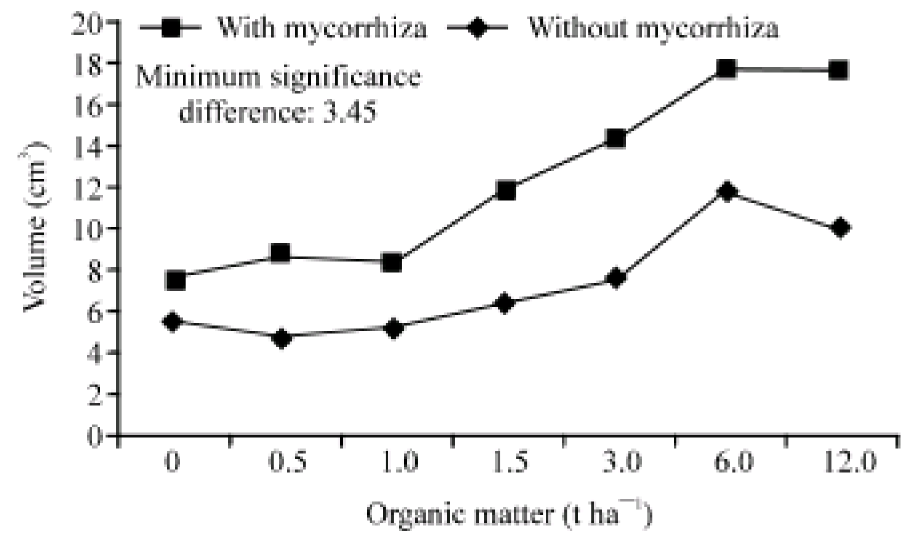

Fig. 4: Effect of arbuscular endomycorrhizae fungi and different levels of organic matter in root volume (cm^3) of *Leucaena leucocephala*

CONCLUSIONS

In general the levels of Copper (Cu), Chromium (Cr), Lead (Pb) and Nickel (Ni) are higher in the soil irrigated with sewage water, almost twice that of the other soil, except for chromium which has a lower level in soil irrigated with clean water.

However, in time the increase of organic matter and high pH in the soil could have toxic effects on grassland and inadequate balance of nutrients. This situation that is not now considered as a problem by the farmers of this area.

The inoculation with *Glomus intraradices* and the application of 6 t ha^{-1} of OM produced the higher value for all evaluated variables (plant height, stem diameter, root volume) of *Leucaena leucocephala*. Previous evidence showed that this plant has higher dependence on this mycorrhizal fungus, because the treatment with this fungus produces the highest values for all evaluated variables.

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