Soil Contamination And Its Effects On Beans (*Phaseolus Vulgaris* L.) Growth Affected By Organic Matter, And Associated With *Glomus Intrarradices*

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Abstract

Contamination is a growing problem affecting irrigation water. The objective of this research was to evaluate the effect of two sources of irrigation water, clean one, sewage water, and organic matter on the association with *Glomus intrarradices* on beans (*Phaseolus vulgaris* L.). The interpolation of the chemical analysis did not find differences between clean and sewage waters regarding the increase of contaminants in the soil. The contamination of the soil by sewage water did not affect plant growth or yield. It only affected nitrogen fixation. There were highly significant increases ($p \leq 0.05$) in all the variables recorded due to the application of organic matter, and to the inoculation with *Glomus intrarradices*. The irrigation source of the soils used in this experiment only had a significant effect ($p \leq 0.05$) on pod number and nitrogen fixation. The best growth and grain yield occurred with inoculated plants and supplementary organic matter.

Keywords: Soil contamination, vermicompost, sewage water, arbuscular endomycorrhiza, edible legumes

Introduction

Common bean is the most important legume in Mexico. Around 1.7 million hectares were planted in 2015 in this country (SIAP, 2016). It is cultivated mainly in areas where water is scarce. Sewage water has been used for its irrigation. Studies on its effect on plant growth and yield are needed.

Farmers usually apply chemical fertilizers. Its cost has been increasing dramatically (Huang, 2009). Organic matter sometimes is also used. However, their consequences on beneficial microorganisms as endomycorrhiza need to be understood. *Glomus intrarradices* has been used to improve plant growth under different conditions included contaminated soils. Several researchers consider that kind of fungi as the most important organisms on earth interacting in agro environments. More than 80% of all terrestrial plants, among them most of the horticultural and crop plants have a symbiotic relationship with these fungi. The stimulation of plant growth can be attributed mainly to the improvement of phosphorus nutrition (Alarcon, 2008; Gardezi *et al.*, 2005, 2008, and 2015; George *et al.,* 1994; Plenchette *at al.,* 2005).

Glomus intrarradices has increased bean yield 36% (Irizar *et al.,* 2003). Novella *et al.* (2003) had reported augmented corn and bean yield when they were cultivated together and were inoculated with a combination of *Rhizobium* and mycorrhiza.

The objective of this study was to map soil contamination due to the use of sewage water and investigate its effect on the growth and yield of beans associated with *Glomus intrarradices*.

Materials and methods

The study was done in agricultural fields, and under greenhouse conditions at the Postgraduate College, Montecillo Campus, State of Mexico, in the spring and summer of 2014.

Two soils, from Tocuila, Texcoco, Mexico, were used. One came from a one-hectare parcel irrigated with sewage water, and the other one was from another one-hectare field irrigated with clean water from a well. Geographical Information Systems were used. Sixteen wells were located using GPS in a digital orthophoto from the Mexican Institute of Geography and Informatics from 1996 with a 2 m pixel, scale 1:20,000.

From the soil samples obtained, a 17 variables chemical analysis, and a 13 variables agronomical analysis were performed. An Excel spreadsheet with the chemical, and the agronomical variables was constructed. It was transformed to Dbase later. The coordinates were transferred to a point map in GIS ArcView v. 3.2 associated to the spreadsheet using the Trigulat

Irregular Network (TIN) method. This allowed analyzing the movement of chemical elements such as potentially toxic heavy metals from the samples.

The treatments were: planting in soil irrigated with sewage water and the other one with clean water. Both soils were collected at three depths 0-5, 5-10, and 10-40 cm from plots of one hectare each (Castellanos *et al*., 2000).

The inoculation was done during the planting, mixing 5 g of sand with sorghum roots with 78 % colonization of *Glomus intrarradices* and 1050 spores per 100 g of inert material. Two levels of *Glomus* were applied, with and without *Glomus*.

Table 1. Soil analysis for the two plots, one irrigated with sewage water and the other with clean one.

Soil sample	pH	EC	OM	TN	NO_3	P	CEC
	1:2	dm sec^{-1}	%	%	mg kg^{-1}	mg kg^{-1}	C mol (+) kg^{-1}
Residual water							
Soil depth 0-5 cm	7.44	349	2.5	0.098	18	15	19
Residual water							
Soil depth 5-10 cm	7.37	454	2.48	0.096	17	14	18
Residual water							
Soil depth 10-40 cm	7.44	475	2.45	0.094	15	11	15
Clean water							
Soil depth 0-5 cm	7.52	314	2.49	0.097	17	14	18
Clean water							
Soil depth 5-10 cm	7.75	332	2.47	0.095	16	13	17
Clean water							
Soil depth 10-40 cm	7.85	384	2.43	0.092	13	10	13

Soil sample	Ca	Mg	K	Na	Fe	Zn	Cu
	mg kg^{-1}	mg kg^{-1}	mg kg^{-1}	mg kg^{-1}	mg kg^{-1}	mg kg^{-1}	mg kg^{-1}
Residual water							
Soil depth 0-5 cm	1250	59	1180	400	5	3	0.5
Residual water							
Soil depth 5-10 cm	1210	56	1220	640	5	3	0.5
Residual water							
Soil depth 10-40 cm	1245	59	1400	800	4	3	0.4
Clean water							
Soil depth 0-5 cm	1240	58	1120	360	5	3	0.5
Clean water							
Soil depth 5-10 cm	1243	60	1110	640	3	3	0.4
Clean water							
Soil depth 10-40 cm	1200	53	1140	720	4	2	0.4

Key: pH= Hydrogen potential, EC=Electrical conductivity, OM= Organic matter, TN= Total nitrogen, NO3= Nitric nitrogen, CEC= Cation Exchange Capacity.

Organic matter was applied as a vermicompost. Four doses were applied. In every bag of three kg, 0, 28.86 g, 57.7 g, and 86.46 g of vermicompost were mixed. They were equivalent to 0, 25, 50, and 75 t ha^{-1} of organic matter.

The variables evaluated were: plant height (PH, cm), leaf area (LA, cm^2), pod number (PN), grain dry weight (GDW, g), root length (RL, cm), root volume (RV cm^3), root dry weight (RDW, g), pod dry weight (PDW, g), grain number (GN) biomass dry weight (BDW, g), white nodule number (WNN), red nodule number (RNN), and total nodule number (TNN).

A factorial arrangement with 16 treatments (4x2x2) was used with a completely randomized block design with three replications. An analysis of variance for all variables registered was done, and a Tukey mean comparison test for the significant variables.

Results and Discussion

Table 1 shows the average values for the chemical analysis of the sixteen wells sampled. No geographical pattern was found for pollutants (Figures 1 and 2). Sewage water only increased the quantity of Cu and Zn, but its levels were below the threshold for considering them as contaminants (Castellanos *et al*., 2000). No Cr or Ni traces were found. The soil texture was sandy loam. Its higher infiltration rate could reduce the contaminant buildup.

The soil pH was alkaline. It was higher in those irrigated with clean water. The difference was greater in the 10-40 cm depth. The soil watered with clean water had a pH of 7.85, and the one with sewage water, only 7.44 (Table 1). The electrical conductivity (EC), organic matter (OM), total nitrogen (TN), nitric nitrogen (NO3), phosphorous (P), Cation Exchange Capacity (CEC), calcium (Ca), potassium (K), sodium (Na), iron (Fe), zinc (Zn), and copper (Cu) quantities were higher in the soils that were irrigated with sewage water.

There were highly significant differences ($p \leq 0.01$) in all the variables recorded due to the application of organic matter, and to the inoculation with *Glomus intrarradices*.

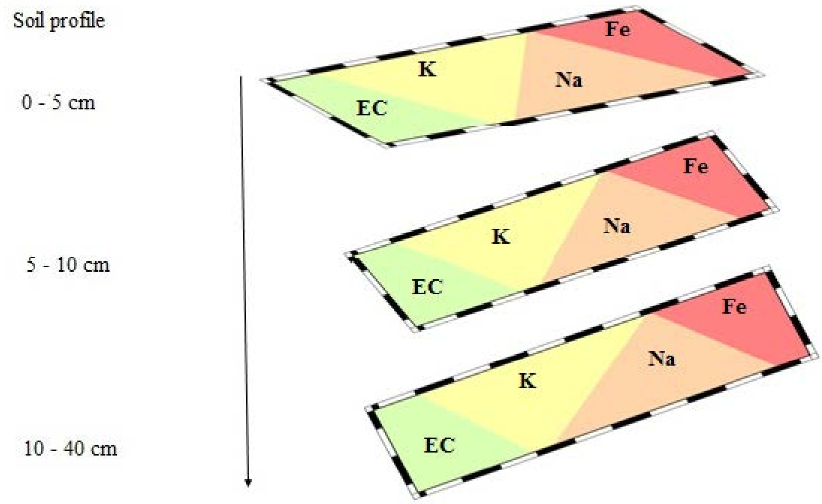

Figure 1. Soil analysis of three profiles irrigated with clean water.
Key: EC: Electrical Conductivity; K: Potassium; Na: Sodium; Fe: Iron.

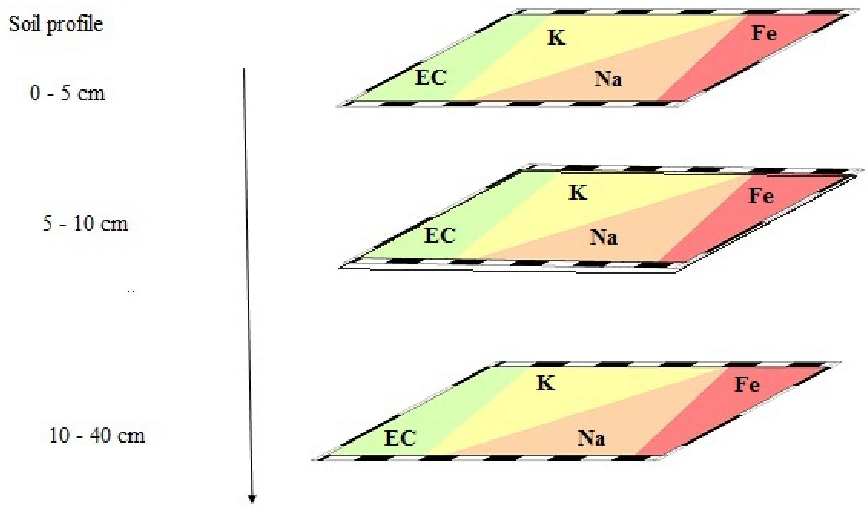

Figure 2. Soil analysis of three profiles irrigated with sewage water.
Key: EC: Electrical Conductivity; K: Potassium; Na: Sodium; Fe: Iron.

As it is shown in Figures 1 and 2, sixteen samples were obtained in order to analyze the movement of chemical elements, such as potentially toxic heavy metals, in different soil depths. The soils irrigated with clean water (Figure 1) did not have any significant difference in electrical conductivity, or K, Na, and Fe content in the three depths (0-5, 5-10, 10-40 cm; Figure 1). A similar situation was found in the soils irrigated with sewage water (Figure 2). There were no elements potentially toxic in those soils. It is advisable to continue monitoring heavy metals in regular intervals to ensure they remain in safe levels.

In both soils, total nitrogen (TN), and the nitric nitrogen (NO3) quantities were low. The distribution was higher in the 5-10 cm layer. No ammoniacal nitrogen (NH4) was found in the two soils. It explains why total nitrogen is only slightly higher on the soils that were irrigated with sewage water that had a greater organic matter (Lavelle and Spain, 2005).

There were highly significant differences ($p \leq 0.01$) in all the variables recorded due to the application of organic matter, and to the inoculation with *Glomus intrarradices*.

The pod number, and the white, red, and total nodule number were significantly affected ($p \leq 0.05$) for the contamination from the soil with sewage water. Their higher content of nitric and total nitrogen could reduce the nodule number. It is well known the antagonistic effect between nitrogen content is soils and nitrogen fixation (Dong *et al*., 2011). The lack of effect on growth and yield could be explained by the low N fixation (Gardezi *et al*. 2005 and 2015; Lindemann and Glove, 2003).

A positive effect of inoculation with *Glomus intrarradices* was found. The highly significant differences ($p \leq 0.01$) among treatments for all the variables recorded generated a beneficial effect on plant growth due to an improvement in the absorption of mineral nutrients required by the plants (Aryal *et al*., 2003 and 2006). This behavior was similar to that found by Gardezi *et al*. (2005, 2008b, 2009, 2010, and 2015).

Table 2. Effect of the inoculation with *Glomus intrarradices* on common bean (*Phaseolus vulgaris* L.).

Glomus intrarradices	Plant height (cm)	Dry weigth aerial part (g)	Leaf area (cm2)	Grain number (ln)	Grain dry weight (g)	Pod dry weight (g)	Pod number (ln)
Inoculated	124.542a	24.292a	448.79a	2.9806a	10.958a	3.0000a	2.1849a
No inoculated	100.667b	16.375b	357.92b	2.3845b	5.083b	2.1667b	1.8475b

Glomus intrarradices	Root lenght (cm)	Root volume (cm3)	Dry weigth root (g)	White nodule number (ln)	Red nodule number (ln)	Total nodule number (ln)
Inoculated	28.708a	9.7917a	3.0833a	1.9818a	2.6397a	3.0620a
No inoculated	19.500b	6.2500b	2.0833b	1.2320b	1.9258b	2.2557b

ln=transformed to natural log. Means with the same letter in each column are not significantly different (Tukey $\alpha = 0.05$).

Yield, root and shoot growth from plants with mycorrhiza were superior to those without inoculation (Table 2). The treatments with mycorrhiza were 54% taller, had 25% more leaf area, 48% heavier shoot dry weight, 47% longer roots, 56% greater root volume, and 48% more root dry weight. This is an indication of a positive effect of mycorrhiza on plant growth originated by better mineral nutrient absorption required by the plant (Alarcon, 2008; Aryal *et al*., 2003 and 2006; Gardezi *et al.*, 2015). Gardezi *et al*. (2007, 2008a, 2009, and 2015) also found this beneficial effect in *Leucaena lecocephala* associated with endomycorrhiza and with Rhizobium. Positive responses to the inoculation with mycorrhiza were also found in a number of species (Gardezi *et al*., 1990 and 2015; Irizar *et al*., 2003; Tawaraya, 2003), including beans (Aryal *et al*., 2003 and 2006; Bermudez *et al*., 1995).

The inoculation with *Glomus intrarradices* improved root and shoot growth. It also had a beneficial effect on the biological nitrogen fixation, and a superior absorption of nutrients (Gardezi *et al.,* 2015). Thus, it contributed to higher yield in beans, coinciding with other studies (Aryal *et al*., 2003 and 2006). Inoculated plants had 38% heavier pods and yielded 116% more grain (Table 2).

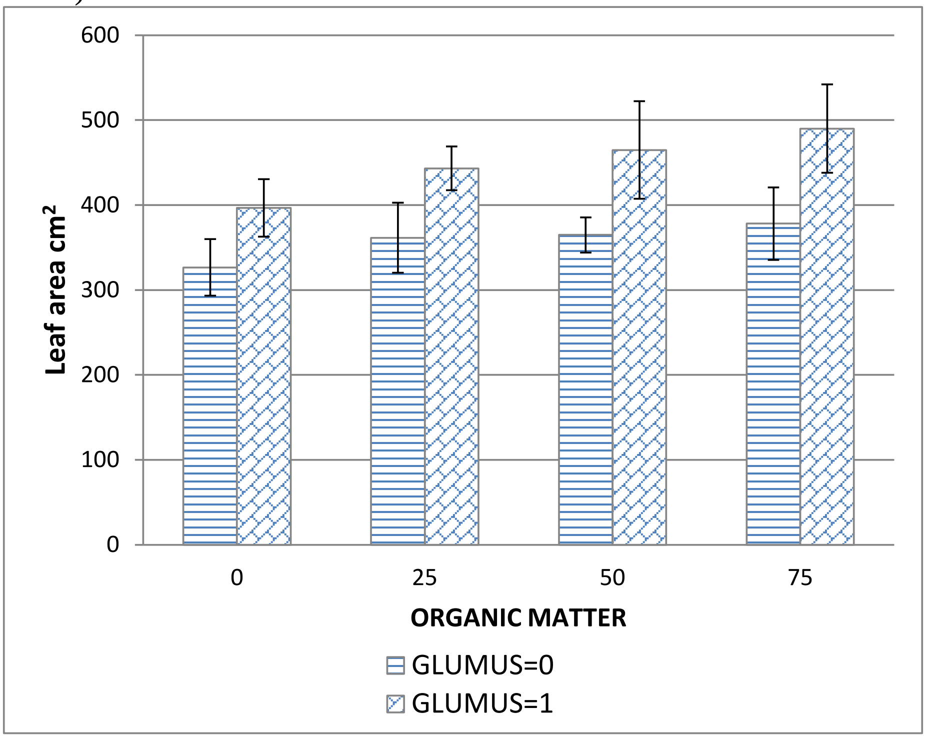

Figure 3. Effect of inoculation with *Glomus intrarradices* and organic matter on soils with two types of irrigation on leaf area of three cultivars common bean (Phaseolus vulgaris). Key: Glumus=0: Noninoculated, Glumus=1: Inoculated with Glomus intrarradices. The vertical lines indicate standard error.

Plant growth was affected by the organic matter application (Table 3). It provided significantly higher ($p \leq 0.01$) plant height in all the treatments compared with the control. Aryal *et al*. (2003 and 2006) found similar results. Only the higher quantities gave heavier dry weight of the aerial part. A similar situation was found in the leaf area (Figure 3).

Root length and root volume were also significantly greater ($p \leq 0.05$) only with the two higher applications of vermicompost. However, all the doses of organic matter gave heavier roots.

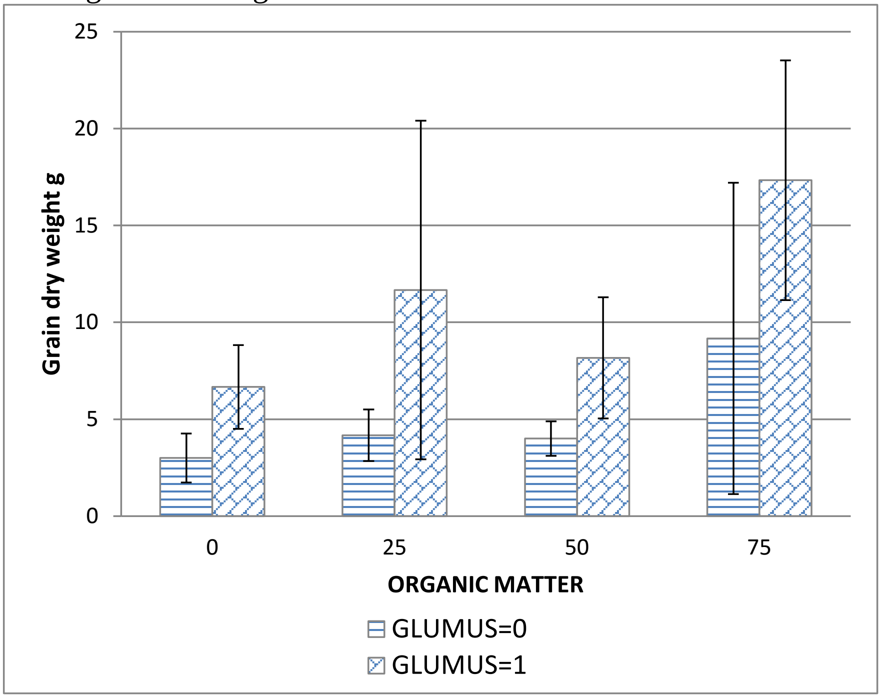

Figure 4. Effect of inoculation with *Glomus intrarradices* and organic matter on soils with two types of irrigation on grain yield of common bean (Phaseolus vulgaris).
Key: Glumus=0: Noninoculated, Glumus=1: Inoculated with Glomus intrarradices. The vertical lines indicate standard error.

Organic matter also promoted nitrogen fixation. All the vermicompost applications had a significantly higher total nodule number. However, only the elevated dose was related with a greater white and red nodule number.

In an analogous way with mycorrhizal inoculation, the organic matter promoted better root and shoot growth. Therefore, as a result, photosynthetic production increased. Pod number was higher with organic matter. Grain yield was 174% enhanced with the highest dose of organic matter compared to the control (Table 3, Figure 4).

Thus, the poorest growth and grain yield occurred with uninoculated that lacked supplementary organic matter (Table 3, Figure 4).

Table 3. Effect of organic matter (vermicompost) on common bean (*Phaseolus vulgaris* L.).

Organic matter $t*ha^{-1}$	Plant height (cm)	Dry weigth aerial part (g)	Leaf area (cm2)	Grain number (ln)	Grain dry weight (g)	Pod dry weight (g)	Pod number (ln)
0	90.167b	15.333b	361.67b	2.289b	4.833b	2.083b	1.824b
25	115.000a	19.833ab	402.50ab	2.511ab	7.917ab	2.667a	1.949ab
50	123.417a	22.917a	415.00a	2.963a	6.083b	2.750a	2.131a
75	121.833a	23.250a	434.25a	2.968a	13.250a	2.833a	2.161a

Organic matter $t*ha^{-1}$	Root lenght (cm)	Root volume (cm3)	Dry weigth root (g)	White nodule number (ln)	Red nodule number (ln)	Total nodule number (ln)
0	19.583b	5.917b	2.000b	1.125b	1.744b	2.070b
25	24.833ab	7.667ab	2.750a	1.637ab	2.564a	2.919a
50	25.917a	9.083a	3.000a	1.682ab	2.337ab	2.692a
75	26.083a	9.417a	2.583a	1.983a	2.487a	2.954a

ln=transformed to natural log. Means with the same letter in each column are not significantly different (Tukey α= 0.05).

The irrigation source of the soils used in this experiment only had a significant effect ($p\leq0.05$) on pod number and nitrogen fixation. The higher content of organic matter and nitrogen found in the soils watered with sewage water generated a greater pod number. However, this effect was not present in grain yield.

The nitrogen fixation, measured as the nodule number (white, red, and total) was higher in the soils irrigated with clean water. A possible explanation for this finding is the antagonistic effect between nitrogen content is soils and nitrogen fixation (Dong *et al.*, 2011). The soils watered with sewage water had higher total and nitric nitrogen (Table 1). Gardezi *et al.* (2012, 2013, and 2015) found similar results in previous experiments.

Conclusion

The soil chemical analysis of each plot allowed to have the distribution of nutrients and contaminants. The interpolation of the chemical analysis did not find differences between clean and sewage waters regarding the increase of contaminants in the soil. The contamination of the soil by sewage water did not affect plant growth or yield. It only affected nitrogen fixation. Mycorrhizal inoculation and nitrogen fixation provided higher bean root and shoot growth and therefore, better yields. The application of organic matter, as vermicompost, improved plant growth, and grain yield.

In a future study, a larger area will be sampled to search for higher variability among clean and sewage water.

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