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Full Length Research Paper

Effects of compost made with sludge and organic residues on bean (*Phaseolus vulgaris* L.*)* crop and arbuscular mycorrhizal fungi density

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In this study we evaluated five composts prepared with two sludge: from a beverage factory and from a paper industry; they were mixture with organic residues from a pepper canned and corn stubble. Composts were tested over yield of common bean and population of arbuscular mycorrhizal (AM) fungi. Mixtures of residues and sludge were composted during four months producing a non phytotoxic material with good physical and chemical properties. Bean cultivated with the application of three composts growth higher than control (without compost). Considering the bean production it was higher in plots applied with composts 1, 2 and 5 compared with control, these show high nitrogen content. In plots applied with the five composts the number of AM fungi spores found was higher than in control. We demonstrate that the use of sludge to make compost is an interesting way to take advantage of residues and a manner to improve the soil properties, because the organic matter and minerals of waste and sludge could be transformed into compounds that could recover the soil fertility.

Key words: Arbuscular Mycorrhizal fungi, compost, bean, sludge.

INTRODUCTION

Agricultural soils are often subject to excessive erosion, nutrient run-off, and loss of organic matter and, consequently, a decline in fertility. One method of reversing the degradation and improving the quality of soils involves the addition of several kinds of wastes such as solid organic waste, sewage sludge, agricultural and industrial wastes, and animal manure (Crecchio et al., 2001). In recent years, application of organic wastes to agricultural lands has received considerable attention owing to the cost and environmental problems associated with their disposal (Ahmad et al., 2006).

Compost has favorable effects on physical and chemical properties of soil, like pH, and the capacity to absorb nutrients from soil, increasing the availability of macro and micronutrients that stimulates micro-organisms development (Biala, 2000). Compost obtained from the organic fraction of municipal solid waste, from sewage sludge and from other selected waste biomasses can be effective fertilizer for agriculture (Zhang et al., 2006).

The aim of this study was to evaluate the use of compost prepared with sludge from a beverage and a paper industry wastewater treatment plants, and to estimate their effect on yield of common bean crop (*Phaseolus vulgaris L.)* and assess it potential impact on the population of arbuscular mycorrhizal (AM) fungi as outcome of the composts application.

MATERIALS AND METHODS

Experimental field location

Study site was established in the municipality of Altzayanca, Tlaxcala, Mexico (19°18'32'' N, 97° 50'47'' WO; about 2416 m above the sea level). Weather conditions are semi desertic, with July to September rainfall. Soils are Dystric Regosols (Werner, 1988; IUSS et al., 2006).

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Table 1. Composition of the mixtures prepared for composting.

	Percent volume (% V)				
Component	**Compost 1**	**Compost 2**	**Compost 3**	**Compost 4**	**Compost 5**
Sludge paper manufacturer	30	0	45	0	45
Sludge soft drink manufacture	30	35	25	0	45
Chili pepper residues	30	55	25	75	0
Corn stubble	10	10	5	25	10

Compost preparation

The composts were prepared by mixing different proportions of not toxic sludge from a waste water treatment plant of a paper mill and a soft drink manufacturer, with a waste from chili preserves, corn stubble was used to improve the porosity and ensure the entry of oxygen (Raviv, 2005). The composition of mixtures prepared for the composts is in Table 1. Mixtures were turned and added water weekly for four months, according to the aerated static pile method (Willson et al., 1980).

Physicochemical analyses

After four months, the composts were homogenized and sieved (sieve 2 mm) for chemical and physical analysis. Determination of total N and pH were measured according to Wu et al. (2000), Organic Matter, Organic Carbon (method AS-07; method Wakley and Black), Electrical Conductivity (method AS-18), Total Nitrogen determination (method AS-25) and determination of the C/N and Phosphorus (method AS-10; Procedure Olsen) (NOM- 021-RECNAT-2000) . The microbiological analysis of the composts was made based on NOM-004-SEMARNAT-2002 standard.

Phytotoxicity test

Maturity of composts was evaluated with the percentage of germination index (% GI) using seeds of lettuce (*Lactuca sativa* L.) following the method reported by Tiquia (2000) who reported that germination index equal or higher than 80%, is an indicator of compost maturity.

Plants growth

Land was prepared by means of mechanical fallow work, tracking and furrowed. Terrain was divided in six plots of 4 m wide by 6 m long. The seeds (*Phaseolus vulgaris* L of region) were put in holes (15 cm depth), at 30 cm from one to another. Composts were added at a rate of 80 g/hole. Control was a plot without compost or fertilizer. Plants height was measured periodically and the beans yield in each plot was calculated. Rhizosphere soil were taken to count the AM spores, sampling was carried out taking into account three phenological stages of plant: seedling stage V4 (Trifolio), flowering (R6 stages) and stage R8 (pod ripe or mature plant) (IICA et al., 2009).

Extraction and counting of AM fungi spores

Spores of AM fungi were isolated from rhizosphere soil samples by the techniques of wet sieving and decanting, and the technique of sucrose gradient centrifugation method (Brundrett et al., 1996). Spore isolation was performed using sieves of 500, 212, 106 and 40 mm respectively, the material retained on each sieve was centrifuged at 3000 rpm for 3 min. Only viable spores were separated and counted.

Statistical analyses

All the results reported in this work were expressed as means of three replicates. Results were analyzed by ANOVA analysis with one factor to find significance statistical difference between treatments, using a significant value of 5%. Tukey test (5%) was used for comparison of means to assess the significance difference in results.

RESULTS AND DISCUSSION

Analysis of physical and chemical properties of the composts

Soil of Altzayanca locality had a sandy texture (60 to 68% sand) characterized by low fertility, with organic matter contents below 2%, alkaline pH (8.0), electrical conductivity of <2 dS/m^{-1} and a high degree of erosion, similar with those reported by Gutiérrez and Ortiz (1999). Table 2 shows the physical and chemical analysis of composts and control soil of Altzayanca.

pH in composts were slightly alkaline (7 to 8.53), however there are some reports indicating that compost made with sludge exhibit alkaline values, as Wu et al. (2000) whose reported final pH 8.3 to 8.4 for composts made with sludge and garden residues; and Iñiguez et al. (2006) who reported pH 8.67 to 8.55 for compost with sludge and agave residues. All compost show good organic matter content, this is the main factor to determine the agronomic quality of (Kiel, 1985).

Mixtures of residues had an initial values C/N ratios of 18 to 32 (Table 1), and final C/N ratios (in composts) from 16.46 to 7.69. Decrease in C/N relations indicates that during composting process the material was degraded and mineralized. The C/N ratios for composts were similar to that reported by Iñiguez et al. (2006), C/N ratios of 11.58 and 11.07 for composts with sludge and agave residues.

In other hand, Moreno et al. (2008) indicated that electrical conductivity has to be lower than 1.50 dS/cm, because a high value shows excessive salt content and this could affect the absorption of water and nutrients by plants (Bhoopaner et al., 2003). Only compost 2 shows

Table 2. Physical and chemical analysis of composts and control.

Parameter (units)	Compost 1	Compost 2	Compost 3	Compost 4	Compost 5	Soil
pH (H_2O)	8.25 ± 0.03	8.53±0.002	7.23 ± 0.03	8.47 ± 0.03	7.00 ± 0.04	7.34 ± 0.06
Organic matter (%)	8.10 ± 0.07	12.9±0.89	8.33 ± 0.19	11.76±0.51	13.33 ±0.51	1.64 ± .2
Total Nitrogen (%)	0.55± 0.08	0.97± 0.07	0.29± 0.07	0.51± 0.09	0.71± 0.08	0.1± 0.02
C/N ratio	8.49 ± 1.4	7.69±1.043	16.46 ±3.06	13.46±2.23	9.66 ± 1.08	9.13 ± 0.12
Phosphorus (kg/ha^{-1})	6.75 ± 0.7	10.75± 0.87	7.61 ± 0.87	9.50 ± 0.38	8.33 ± 0.95	0.42 ± 0.64
Electric conductivity dS/cm	0.88	1,67	1.12	1.54	1.35	0.04
Helminthes eggs (HE/2gTS)	< 1	< 1	< 1	<1	< 1	
Fecal coliforms (MPNC)	23	430	230	<3	210	
Salmonella spp (MPN/gTS)	< 3	< 3	< 3	<3	< 3	

pH in water 1:2, HE/2gTS, Helminthes Eggs per total soil gram, MPNC, most probable number of coliform, MPN/gTS, most probable numbers per total soil gram.

an electrical conductivity higher than this value. Additionally content of Phosphorous were better for composts compared with the soil of the Altzayanca region. Microbiological analysis indicates that during composting process, the temperature rise was enough to kill pathogen microorganisms (like *Salmonella* spp), helminthes eggs and fecal coliforms. Sludge and waste were transformed into material with good characteristics to apply in soil (Table 2).

Phytotoxicity test

Use of compost has been increased in recent years either as a soil conditioner or as component base for the development of specialized agricultural substrates. After four months, all % GI were 80% and above guarantying assuring the biological stability and maturity of entire composts.

Plant height

Plants growth was similar in plots with compost addition and the control (Figure 1) . Tukey test (0.05%) shows that there were no significant differences in height measured between plot treated plants and plot control plants.

Crop yield

Application of the composts 1, 2 and 5 improve the yield comparing with the control plot (Figure 2). Highest production was reached in the plot applied with compost 5, with yield of 337 kg ha that represents 91% of yield reported by Copladet (2005). Second yield was for plot treated with compost 2 and the third was using compost 1 with a yield of 216 and 146 kg ha^{-1}, respectively. While with composts 3, 4 and control the yields were about 120 kg ha^{-1}. Composts 5, 2 and 1 were made with the high content of sludge from the beverage factory; Those compost had high content of nitrogen compared with sludge from the paper factory (1.29 and 0.36% N, respectively). Bean crop required high concentrations of N, K and Ca (Guedes and Junqueira, 1978) and low quantities of P, Mg and S for healthy growth. In addition compost 5 had neutral pH, the major quantity of organic matter and good amount of % N and % P, this explain the high beans yield observed. Whereas compost 2 had 12.9% of organic matter and the highest quantity of % N and % P, however the pH (8.53) was slightly alkaline and decreases the availability of nutrients and the bean yield. Low yield in plot with compost 1 was due to its high % N and the low % P and alkaline pH.The low beans yield with composts 3 and 4 were either due to poor % organic matter and % P and high pH. In all cases the beans yield were lower than that reported by Copladet (2005) however results are after only one application of composts.

After application of composts and bean production, the soil improves in the % C, % Organic matter, % N and % P compared with initial values, indicating that composts provided nutrients to the soil besides to the plants. The pH remained near neutrality and electrical conductivity increased too, because of minerals salts present into the composts (Table 3).

Economical analysis

In 2009 the price per ton in Mexico of urea and triple phospate fertilizers was 248.3 and 235.7 US dollars, respectively (Navarrete, 2010), and the compost was 84 US dollar per ton (Fenifos, 2009) . In the Altzayanca region the fertilization is made with a mixture of 200 kg urea and 100 kg triple phosphate per hectare. Considering this quantities the application of fertilizer in this work cost 72.23 US dollars per hectare, low-priced than compost. Even thought subsequent aplications of compost could improve the physical and chemical

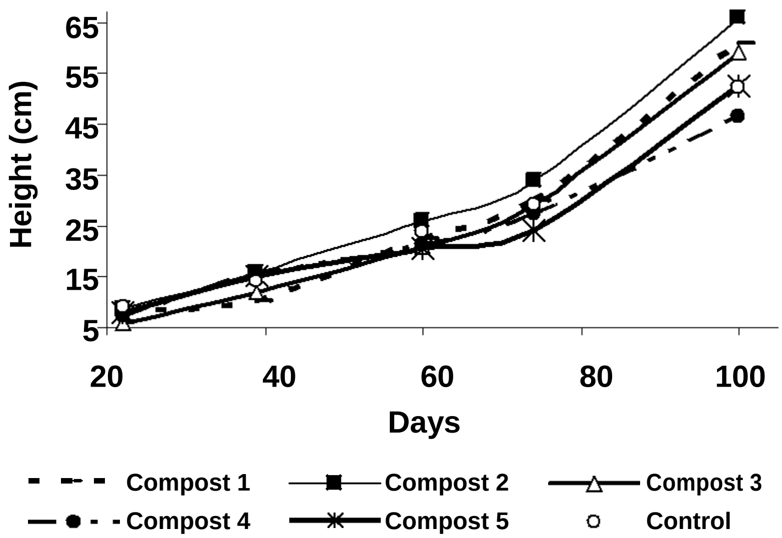

Figure 1. Plant height of treatment plots.

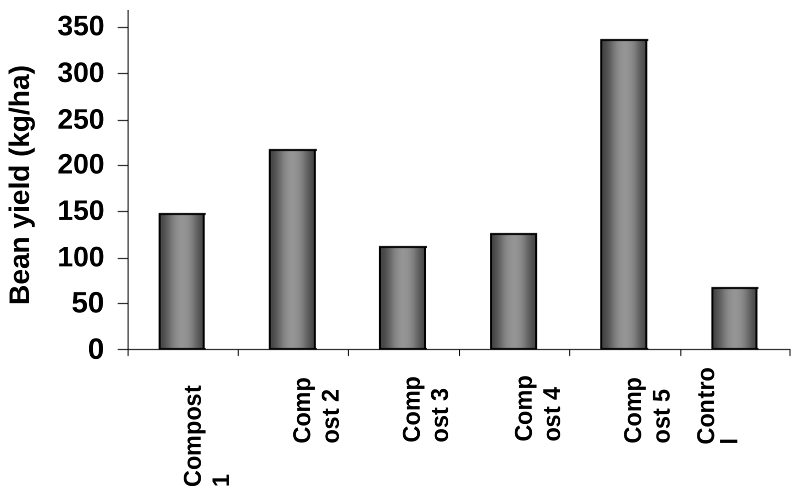

Figure 2. Beans Crop yield on the treatment plots.

Table 3. Physical and chemical parameters of soil before and after the treatments.

Soil before application of composts							
Parameters	**pH**	**EC (dS m^{-1})**	**OC (%)**	**OM (%)**	**TN (%)**	**C/N**	**P (mgkg^{-1})**
Soil	7.34	0.04	0.945	1.63	0.103	9.133	9.385
Soil after a cycle of agricultural production of bean							
COM 1	7.05	0.19	1.18	2.04	0.29	4.01	19.93
COM 2	7.05	0.22	1.70	2.94	0.27	6.40	55.33
COM 3	7.05	0.13	1.36	2.34	0.18	7.51	22.99
COM 4	7.03	0.14	1.40	2.41	0.15	9.21	31.20
COM 5	7.03	0.17	1.51	2.61	0.18	8.37	35.60

pH in water 1:2, EC = Electrical conductivity, OM = organics matter, P = phosphorus, OC = organic carbon, TN = total nitrogen. Data are reported as the mean values range for three replicates.

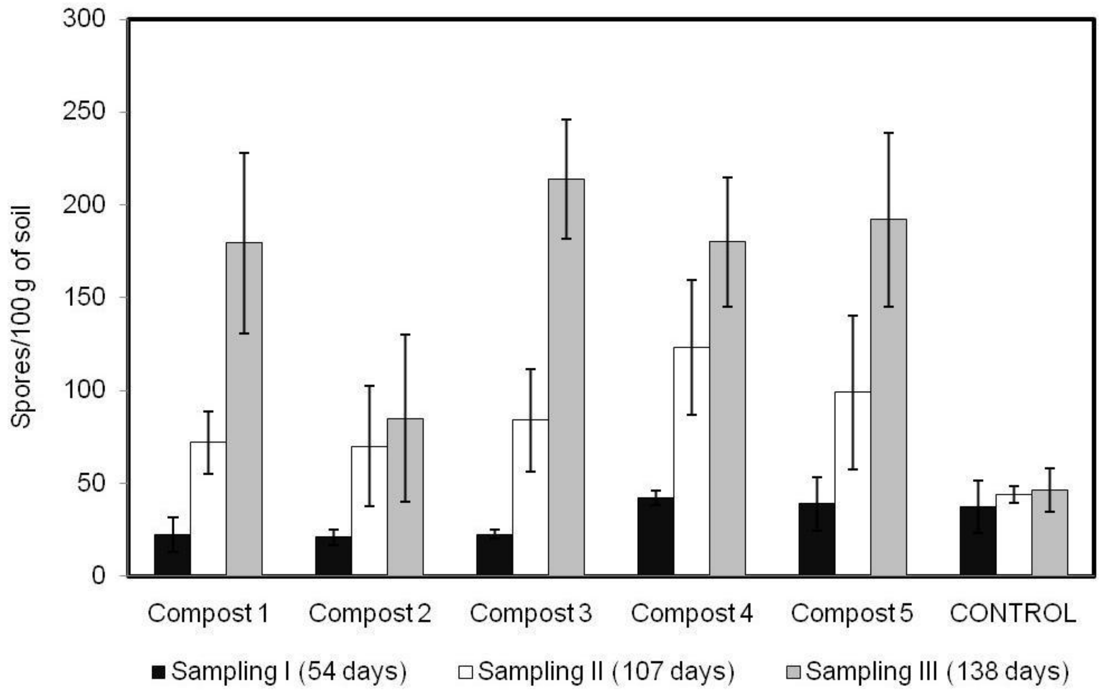

Figure 3. Spores density of AM fungi on plots treated.

properties of the soil, such a way the requirements of compost could be lower with time and eventually economically compared with fertilization.

Density of spores AM fungi

Rhizosphere soil was analyzed in different stages: at seedling stage V4 (Trifolio) the number of spores found in plots applied with compost and control was very low and did not have a statistical significant difference. At stage R8 (pod ripe or mature plant) the higher number of viable spores was present in the plot applied with the compost 3 (214 viable spores/100 g soil), while in the plots with the composts 1, 4 and 5 the number of spores did not have a significant difference between them (179, 180 and 192 spores, respectively) (Figure 3). In all cases spores number were different significatively ($P < 0.05$) from those found in the control plot, indicating that there was a positive correlation between soil fertility and the spores number (Mendoza et al., 2002).

Raise in spore number was according with reports of López- Sánchez and Honrubia (1992), they found that spores number increase according with the plant phenological stage, reporting the maximum density of spores during the fruiting stage in Poaceae, Fabaceae, Asteraceae and Chenopodiaceae families. Also in a previous study with common beans and broad beans in Tlaxcala, the number of spores show relation with the plant phenology with the phenology of the plant, found low quantities sowing at time of planting, and increases during the (Luna-Zendejas 1997). Cousin et al. (2003) showed that the sporulation of different AM fungi were clearly seasonal and were influenced by host plant phenology.

Composts employed had pH from 7 to 8.53, Porter et al. (1987) indicate that pH between 5.5 to 6.5 have positive effect on distribution and abundance of different fungal species as *Glomus* and *Acaulospora*. However, Abbott and Robson (1991) explained that increases in pH and soil nutrient content are related to a decrease in spores density of AM fungi. In contrast Mendoza et al. (2002) and Escudero and Mendoza (2005) indicated that enhance in the pH support fungi the development. In phosphorous case, augment in phosphorous concentration results in a gradual decrease of spore's number affecting root colonization (Deepak, 2008). In soybean fields, spores density of AM fungi had been negatively correlated with the content of phosphorous applied (Isobe et al., 2008). In this work, except for compost 1, it was observed a negative correlation between % P in composts and number of viable spores found.

Conclusions

Sludge and organic matter, could be mineralized through the composting process, resulting in a non-phytotoxic material, where components were transformed and assimilated by crops, improving the beans yield, growth of plants and the population of AM fungi. The use of sludge from wastewater treatment plants is an interesting way to employ their nutrients for the production of a material with good physical and chemical properties; it could be used to reduce environmental problems such as erosion, as a consequence of the continued application of compost.

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