

Full Length Research Paper

Effect of compost made with sludge from wastewater treatment plants on field of corn (*Zea mays* L.) and arbuscular mycorrhizal fungi density

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Accepted 19 May, 2018

In this study we evaluated four composts prepared with different proportion of sludge from two wastewater treatment plants, a soft drink and a paper industry, mixture with and without chili residues. They were tested over yield of corn and population of arbuscular mycorrhizal (AM) fungi. Sludge was composted producing a non phytotoxic material with good physical and chemical properties. Corn cultivated with the application of the four composts grow higher than control (without compost) and in fertilized plot. Considering the corn production it was higher too in plots applied with composts 2, 3 and 4 than in control, and it was similar with those obtained in fertilized plot. There were significant differences in the number of Arbuscular mycorrhizal fungi spores found in samples of soil with application of compost, comparing with control and fertilized plots; application of composts improve the growth and yield. Making compost with sludge from wastewater treatment plants is an excellent way to valorize the residues, because it organic matter and minerals could be transformed into compounds that even could recover the soil fertility.

Key words: Arbuscular mycorrhizal (AM) fungi, compost, corn, sludge.

INTRODUCTION

Nowadays, huge quantities of industrial residues are produced, most of them contains material that could be transformed into a useful products. Various alternatives have been proposed to treat organic waste, like composting. It is one of the main options for reducing large amounts of organic waste. This process lays in the biological degradation of organic matter. During composting organic wastes decompose under controlled conditions (temperature, humidity and aeration). The compost, product of this process, is used as a source of nutrients for sustainable agricultural productivity (Abad

and Puchades, 2002; Eghball, 2002; López and López, 2004; Widman et al., 2005). It has been observed that use of compost increases the organic matter content, providing macronutrients such as N, P, K and micronutrients, thereby improving the cation exchange capacity of the soil. Moreover, the biological activity increases as a result of the application of compost as a source of energy and nutrition for the microorganisms in the soil. The physicochemical properties of soil are also improved through it application (Eghball et al., 2004), contributing positively in the formation and stability of its aggregates, and therefore increases the water and gas permeability, increasing the water retention capacity of this (Caravaca et al., 2002) . Sludge from wastewater treatment plants have been considered as hazardous waste, however, when they are subjected to a process of stabilization by anaerobic biological degradation, they can be exploited beneficially; it contains mineral nutrients, a low presence of pathogenic microorganisms and heavy metals permissible, conferring an agronomic quality; so it can be used as fertilizer or soil conditioners or enhancers

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Abbreviations: C, Carbon; Ca, calcium; CEC, cation exchange capacity; COM, compost; K, potassium; Mg, magnesium; N, nitrogen; Na, sodium; NH₄, ammonium; NO₃, nitrate; P, phosphorous; PPM, parts per million; TSP, triple super phosphate.

Table 1. Composition of the mixtures prepared for composting (%V).

Component	Percentage volume(% V)			
	COM 1	COM 2	COM 3	COM 4
Sludge paper manufacturer	30	45	-----	45
Sludge soft drink manufacture	30	25	-----	45
Chili pepper residues	30	25	75	-----
Corn stubble	10	5	25	10
Initial C/N ratio	21	32	21	20

(Castrejon, 2000).

The fertility of agricultural soil is closely related to microbial populations and the incorporation of organic matter, which brings an increase in the activity of microbial populations by promoting additional benefits related with soil physicochemical characteristics (Triano et al., 2005). In recent years it has promoted the use of other alternatives that help to increase agricultural production and also protect the environment through natural biological interactions of soil biota. In other hand plants through their root system provide a niche for microorganisms that inhabit the soil, like fungi of the phylum Glomeromycota (Schü ler et al., 2001), they form arbuscular mycorrhizal (AM) and mutualistic symbiotic associations are established between their mycelium and the roots of various plants (Klironomos et al., 2001). Once established, AM fungi enhance root mineral nutrition, especially phosphorus, and favor plant growth. Moreover, AM fungi may protect plants against environmental stress such as soil salinity (Klironomos et al., 2001) and drought (Al Karaki et al., 2004).

The aim of this study was to evaluate the employ of compost prepared with sludge from a soft drink and a paper industry wastewater treatment plants, and to evaluate their effect on yield of corn (*Zea mays* L.) and assess it potential impact on the population of AM fungi as outcome of the composts application.

MATERIALS AND METHODS

Field site

The 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). Its climate is semi-dry, with rainfall in the months July to September. Soils are Dystric Regosols (Werner, 1988; IUSS, 2006).

Compost preparation

Composts (COM) were prepared mixing sludge from two wastewater treatment plants, one manufacturer of paper and other of soft drinks; chili pepper from a packing company and corn stubble as conditioner material, to provide structure, porosity and texture necessary to enable aerobic conditions. The composts were done by aerated static pile method (Willson et al., 1980). The piles were turned and added water weekly during four months. The composition of the mixtures of sludge and residues prepared for composting is shown in Table 1.

Physicochemical analyses

After four months composts were homogenized and sieved (sieve 2 mm) for chemical and physical analysis. Determination of total N and pH were measured according to PROY-NMX-FF-109SCFI-2007 (SAGARPA, 2007), organic matter as the NMX-AA-021-1985 (SEMARNAT, 1985a) and phosphorus by NMX-AA-094-1985 (SEMARNAT, 1985b), methods similar with those reported by Wu et al. (2000). The microbiological analysis of the composts was made based on the NOM-004-SEMARNAT-2002 (SEMARNAT 2002).

Phytotoxicity test

Composts maturity was evaluated with the percentage of germination. Tiquia (2000) report that percentage of germination equal or higher than 80%, is an indicator of the maturity of the compost. Two grams of each compost, previously dyed at room temperature, were added to 10 ml of deionized water and kept under magnetic agitation for 1hour at room temperature; the mixtures were filtered through filter paper. In Petri dishes were placed 10 seeds of lettuce (*Lactuca sativa* L.) on a disk of filter paper, it was moistened with the filtrate of the composts. The Petri dishes were incubated at 27°C for 4 days in a chamber (BOD incubator model 208 HACH). The control sample was prepared by using deionized water to moisture the disk of filter paper. During 4 days it was counted the number of seeds germinated per box. Results were expressed as the percentage of germination.

Corn growth

Six plots of 4 m wide by 6 m long were used for experimentation. Land was prepared by mechanically fallow work, tracking and furrowed. The seeds (*Zea mays* L. of region) were put in holes (15 cm depth), at 30 cm from one to other. Composts were added by sowing at a rate of 80 g/hole. A fertilization plot was set, the fertilization was carried out using a mixture of urea and triple super phosphate in a 2.5:1 ratio respectively (250 kg urea/100 kg TSP/ha⁻¹); control was a plot without any addition of compost or fertilizer. Each one of the four composts were applied in one plot: treatment 1 (COM 1), treatment 2 (COM 2), treatment 3 (COM 3), treatment (COM 4). Samples of rhizosphere soil and roots of the crop were taken in three plants selected at random from each plot. Sampling was conducted in three phonologies steps of the plants: seedling stage, in the fruiting stage and in the mature fruit. Also evaluation of the growth of corn was performed by measuring the height of plants in each one of the treatments and after the agriculture cycle it was measured the corn production in each plot.

Extraction and counting of arbuscular mycorrhizal fungi

The AM fungi spores were isolated from the samples of rhizosphere

Table 2. Physical and chemical analysis of composts, control and fertilized soil.

Parameter (unit)	COM 1	COM 2	COM 3	COM 4	SOIL	Fertilized soil
pH	8.25 ± 0.03	7.23 ± 0.03	8.47 ± 0.03	7.00 ± 0.04	7.34 ± 0.06	7.34 ± 0.06
Organic matter (%)	8.10 ± 0.07	8.33 ± 0.19	11.76 ± 0.51	13.33 ± 0.51	1.64 ± .2	1.64 ± 0.2
C/N Ratio	8.49 ± 1.4	16.46 ± 3.06	13.46 ± 2.23	9.66 ± 1.08	9.13 ± 0.12	8.63 ± 0.12
Phosphorus (kg/ha ⁻¹)	6.75 ± 0.7	7.61 ± 0.87	9.50 ± 0.38	8.33 ± 0.95	0.42 ± 0.64	20 ± 1.05
Electric conductivity	0.88	1.12	1.54	1.35	0.04	0.04
Helminthes eggs (HH/2gST)	< 1	< 1	< 1	< 1		
Fecal coliforms (NMPC)	23	230	< 3	210		
Salmonella spp. (NMP/gST)	< 3	< 3	< 3	< 3		

Data are reported as the mean values ± range for three replicates.

soil by the wet sieving and sucrose density gradient centrifugation technique (Brundrett et al., 1996), where 100 g of soil were rinsed through 500, 212, 106 and 38 mm sieves; soil material retained in each sieve was suspended in water and centrifuge at 3000 rpm for 3 min. Only viable spores were recovered one by one under the dissecting microscope and separated and finally they were counted.

Statistical analyses

All the results reported in this paper were expressed as means of three replicates. Results were analyzed by ANOVA analysis with 1 factor to find or not significance difference between composts, fertilized and control plots using a significant value of 5% and using the Tukey's test (5%) for comparison of means to assess the significance difference in results.

RESULTS AND DISCUSSION

Analysis of physical and chemical properties of the composts

The soil of the locality of Altzayanca had a sandy texture (60 to 68% sand), characterized by low fertility, with organic matter contents below 2%, alkaline pH (8.0) and electrical conductivity (<2 dS/m⁻¹) and have a high of erosion, according with (Gutierrez and Ortiz (1999).

The pH values in composts were light alkaline, from 7 and 8.47, however there are some reports indicating that compost made with biosolids reported such values of pH, like Tongetti et al. (2007) reported values for the same parameter from 8.3 to 8.4 for composts prepared with biosolids and municipal organic residues.

Organic matter is one of the most important factors to determinate the agronomic quality of a compost (Kiehl, 1985). All compost show good organic matter content. The initial C/N ratio of the mixtures of residues was between 20 to 32 (Table 1), C/N ratios in composts indicates that during the process of composting the material was degraded and mineralized. The compost C/N values reported in Table 2 were similar to others, Tognetti (2007) reported relations of 11, 13 and 14 for compost made of biosolids with municipal organic waste. Also composts reported good content of P and electrical

conductivity; all results allow noticing that sludge was transformed in material with better physical and chemical characteristics compared with the soil of the Altzayanca region.

During the composting process temperature rise in a good manner (data not shown), such a way the final composts approve the microbiological analysis, helminthes eggs, fecal coliforms and salmonella were in permissible values (Table 2).

Phytotoxicity test

The compost is used today either as a soil conditioner or as component base for the development of specialized agricultural substrates, by this reason assuring of its biological stability and maturity is crucial for it use. A non controlled process results in the production of phytotoxic substances; therefore it is important to confirm the maturity of materials like composts, before to use it in agriculture (Tiquia 2000). After four months all composts showed percentage of germination values >80% (data not shown). These results allow to verifying that sludge from the two wastewater treatment plants in mixtures with or without organic residues (chili), had a good degradation process, producing materials with an adequate mineralization and non phytotoxic, that permit the germination of the lettuce seeds.

Corn growth

Figure 1 shows the growth of corn; it appears that the plants growth in plots with the application of composts was higher than in the fertilized and control plots. The Tukey test (0.05%) shows that there were no significant differences in growth measured with the application of the four composts, and there was significant difference with the growth registered in fertilized and control plots. It is interesting to note that the COM 1 was prepared with both sludge and organic waste (chili), COM 2 with sludge from beverage factory and chili; COM 3 was prepared

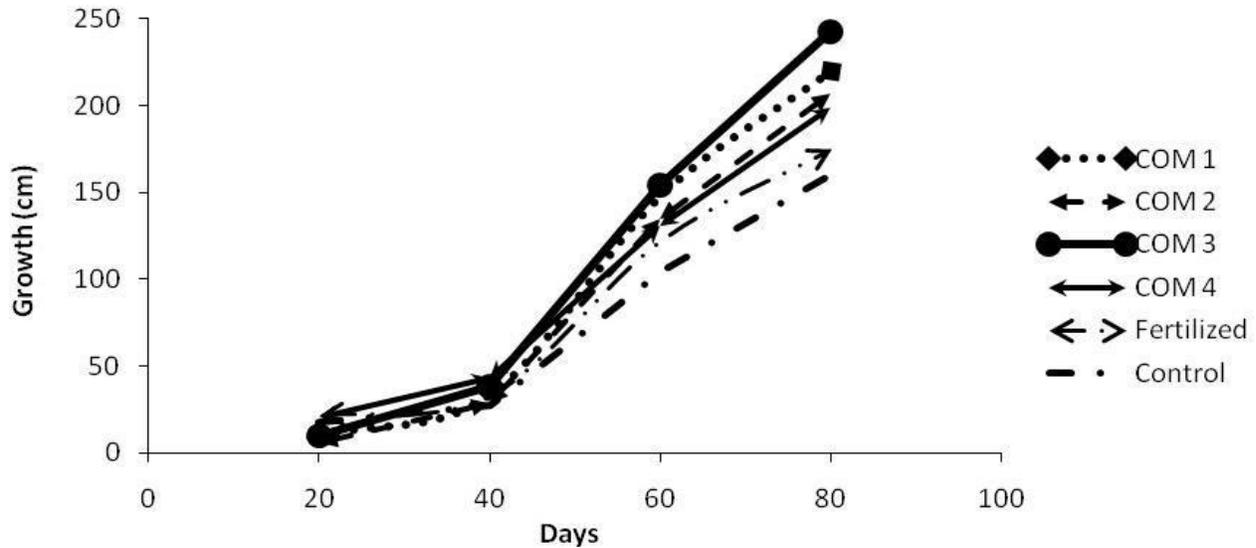


Figure 1. Growth of corn in experimental plots amended with compost prepared with mixing sludge, organic waste (chili) and corn stubble, and unfertilized and fertilized control.

only with organic waste from chili and corn stubble, while the COM 4 was prepared with both sludge and corn stubble and no organic residues (Table 1). Composts made with sludge mixture with other sludge or mixture with organic residues results in a good material that improved the growth of corn.

The observed growth with the addition of composts could be explained due to the physical and chemical parameters of composts (Table 2). The COM 1 had a regular contribution of organic matter (8.1%) and P (6.75 kg ha⁻¹), good C/N relation (8.49) and the pH (8.25) let the availability of the nutrients. COM 2 had a regular organic matter (8.33 %) and P (7.61 kg ha⁻¹) too; however the ratio C/N (16.46) was the highest at the beginning and at the end of the composting process, although the pH (7.23) contributes to the availability of the nutrients. COM 3 had a good organic matter (11.76 %), the best P content (9.50 kg ha⁻¹) and a high C/N relation (13.46), although the pH could affects the availability of the nutrients (8.47). COM 4 has a good organic matter (13.33 %), good C/N (9.66) relation and content of P (8.33) and the neutral pH contribute to the availability of the nutrients. The electrical conductivity was good in all cases, COM 3 (1.54 dS/cm) was in the limit indicated by Moreno (2008), who explains that the electrical conductivity had to be lower than 1.50 dS/cm because when the soil had a higher quantity of salts the adsorption of water by plants is diminished. The incorporation of compost as a soil amendment increased cation exchange capacity (CEC) (Abdelbasset et al., 2009), providing the ability to retains nutrients more effectively (Stofella, 2001). Agricultural amendments as pruning waste compost and biosolids can also enrich the content of P in the soil, for direct input and alteration of the absorptive capacity of P in the soil. The organic P in compost is

easily decomposed to release orthophosphate, available for plants (Stofella, 2001).

In the fertilization treatment, growth was lower than that observed in plots with compost (Figure 1); although the soil had a pH value near to neutral (7.34), which facilitates greater nutrient availability. With the addition of the fertilizer the nutrients surpassed the other treatments with compost application; although it was observed that the soil of this plot was compact, contrasting with those in which the compost was applied. Soil physical properties also play an important role making that the nutrients are retained and available to the plants. The soil in the Alzayanca region is poor in organic matter, it texture does not let the retention of fertilizer applied as salt, by this reason it is easy to lost ions by washing or leaching. Anions like NO³ and cations (NH₄⁺, Ca⁺², Mg⁺², K⁺, Na⁺) could not be retained, excessive water or rainfall favor the drag of to lower strata of the soil away from roots (Castellanos et al., 2000). In the sandy soil texture is favored leaching losses of salts, because the infiltration rate is usually very high, and the drag of nitrate as well (Berti and Cunningham, 2000).

In the control plot was observed that plants showed the lowest growth, because the soil without compost or fertilizer application was poor in nutrients, it is characterized by an almost neutral pH (7.34), a low concentration of N, the contribution of P, organic matter and electrical conductivity parameters were the lowest compared with other treatments.

Yield of corn

Figure 2 shows the yield of corn, the application of the four composts improve the yield comparing with the

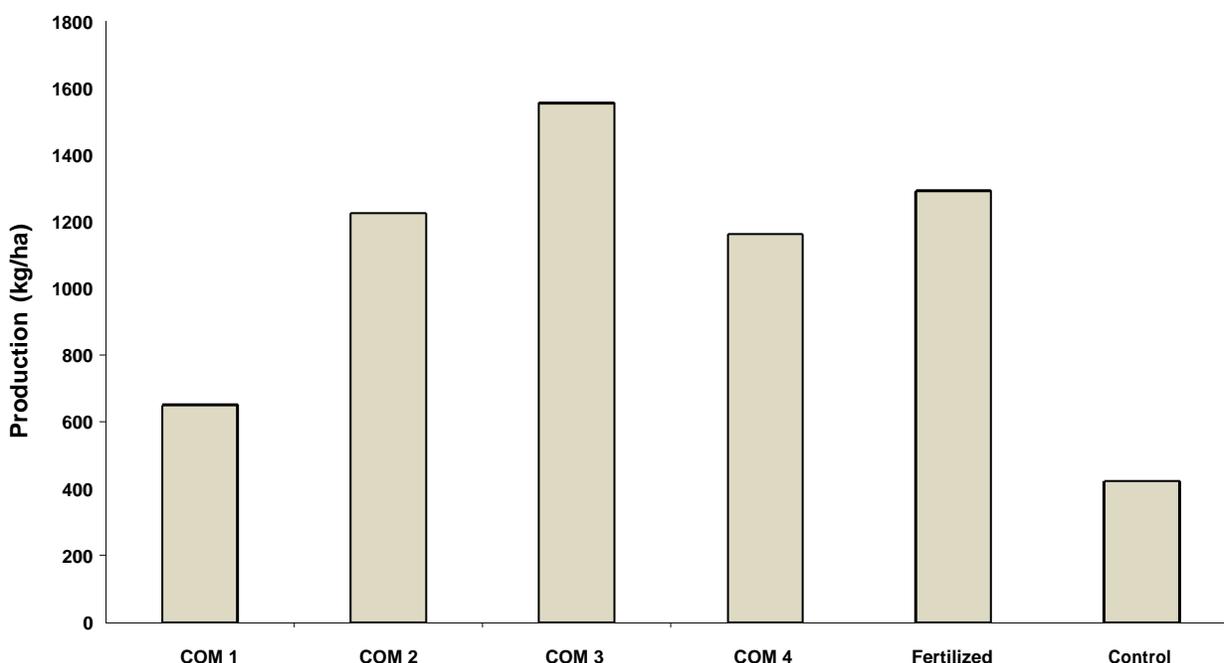


Figure 2. Corn yield in the plots experimental amended with compost prepared with mixing sludge, organic waste (chili) and corn stubble, and unfertilized and fertilized control.

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

Before application of compost							
Parameter	pH	EC (dS/m ⁻¹)	OC (%)	OM (%)	TN (%)	C/N	P (mg/kg)
Soil	7.34	0.04	0.945	1.63	0.103	9.133	9.385
After a cycle of agricultural production of corn							
COM 1	7.99	0.18	1.29	2.22	0.23	5.64	27.14
COM 2	7.89	0.17	1.71	2.95	0.22	7.82	37.40
COM 3	8.05	0.22	1.67	2.88	0.33	5.02	34.70
COM 4	7.07	0.15	1.38	2.39	0.24	5.82	21.72
Fertilized	6.59	0.12	0.61	1.06	0.12	5.08	61.87

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.

control plot. The highest production was reached in the plot applied with COM 3 with an output of 1554 kg ha⁻¹, while treatments with fertilization, COM 2 and COM 4 showed a very similar production (1291.6, 1162.5 and 1225 kg ha⁻¹ respectively), however, productions were even greater than those observed in treatments with COM 1 and control (650 and 422.5 kg ha⁻¹ respectively). The yield in the fertilized plot, adding COM 2 or COM 4 had no significant difference. With the implementation of the COM 3 it was reached higher production than with fertilization; this compost was made using 100 % organic residues without sludge, by this reason residues were completely biodegraded making a good material for plants. With COM 1 the corn yield was smaller than using

the others, this could be explained because that compost had the lower organic matter, lower P and lower C/N relation; it seems like the nutrients of this compost were enough only to growth the plants and not for the fructification of the corn.

Evaluation of physical and chemical properties of land after crop

Table 3 presents the physical and chemical parameters of soil before and after the corn agricultural cycle of production. After the application of composts and the corn production, the soil improve in the parameters:

contain of organic matter was higher indicating that the compost provided nutrients to the soil besides to the plants: The pH remained near neutrality, electrical conductivity increased because of minerals salts into the composts; a similar pattern was observed for the percentage of organic C, which increased as well. It is known that some of the important elements for crops are nitrogen and phosphorus; these were also increased after compost application compared with initial soil. Whereas in plot added with fertilizer, after the cycle of agricultural production of corn, pH was near to neutral, electrical conductivity, nitrogen and phosphorous increased because of the addition of the fertilizer (urea and triple phosphate). In other hand organic matter and percentage C decrease compared with soil before the cycle.

Economical analysis

In 2009 the price of urea in México was 248.3 US dollars and the price of triple phosphate was 235.7 US dollars per ton (Navarrete, 2010). In the Alzayanca region the normal application is 2 parts of urea by one part of triple phosphate, in proportion of 300 kg of mixture of fertilizers by hectare. The application of fertilizer cost 72.23 UD dollars per hectare. In other hand compost price is 84 US dollar per ton (Fenifos, 2009). Application of fertilizer was low-priced than compost, however using compost could be possible to improve the physical and chemical properties of the soil with subsequent applications, such a way the requirements of compost could be lower with time.

The use of compost provides many benefits: waste materials are recycled, reduced the cost of storage and handling of them, take advantage of nutrients as C, N, P and mineral salts from materials. Compost is a substances more available to crops, it successive use, could corrected environmental problems like erosion by, providing organic matter, that improves with time physical and chemical properties of soil with time.

Density of AM fungi spores

The first rhizosphere soil sampling was carried out at 54 days of planting the crop, the number of AM spores found in the experimental plots including fertilized and control, was very low and did not had a significant difference from one plot to other.

During the second sampling, at 138 days in the fruit stage, quantities of AM spores increased in all treatments applied with the composts. The higher number of spores was found in the plot applied with the COM 3 (112 viable spores/100 g soil), while in the plots with the COM 2, COM 1 and COM 4 were 73, 67 and 66 viable spores respectively. Statistical analysis shows that there was no significance difference ($P > 0.05$) in the number of AM

spores found in plots applied with composts, and they differ significance ($P < 0.05$) from those found in the control and fertilized plots. In plots with fertilization and control, the number of spores was the lowest (35 viable spores/100 g soil).

At 172 days, in mature fruit stage, it was recorded the maximum number of spores in all the plots amended with composts, in control and fertilized ones. In plot with the COM 2 was quantified 222 viable spores (by 100 g soil) followed by plots with COM 3, COM 1 and COM 4 (170, 155 and 133 spores respectively). Statistical analysis shows that there was no significance between the number of AM spores found in soil with composts and shows significance difference ($P < 0.05$) comparing with that found in fertilized and control plots. The last had the fewest (26 and 40 viable spores/100 g soil) (Figure 3). There was a positive correlation between soil fertility and the number of AM spores according to Mendoza et al., (2002).

Several authors have mentioned that the pH have an effect on the distribution and abundance of different species of fungi (Porter et al., 1987a; Porter et al., 1987b). According to Abbott and Robson (1991) increases in pH and soil nutrient content are related to a decrease in the density of spores of AM fungi. In contrast some other authors, like Mendoza et al (2002) or Escudero and Mendoza (2005) indicate that an increase in the pH support the development of fungi in this work all the composts had a higher pH compared with the soil before compost application.

The low quantity of spores found in the fertilized soil could be explained because the use of agricultural inputs such as phosphate fertilizer, can decrease the amount of spores of fungi from the rhizosphere of plants, as Covacevich et al. (2005) found in wheat crops, mycorrhizal colonization decreases when the P content exceeds 11 ppm, while applying 5 kg ha⁻¹ the development of mycorrhizal colonization was optimized. Such increase in P concentration results in a gradual decrease of root colonization, which is reflected in the decrease of the number of spores as (Deepak, 2008) reported. In soybean fields the density of spores of AM fungi had been negatively correlated to the content of P applied. In other hand fungal colonization was correlated positively with the spore density and negatively with the content of available phosphorus (Isobe et al., 2008). In this work the soil applied with fertilizer had the higher value of P; this could be the partial reason why in fertilized plot the number of spores of AM fungi found were very low.

Additionally increase or decrease in the number of spores of fungi is correlated with the host plant phenology, as López-Sánchez and Honrubia (1992) found that the maximum density of spores was presented during the stage of fruit (summer), with lower numbers in winter, while in spring tended to increase and remained high during the fall. Also we found in a previous study

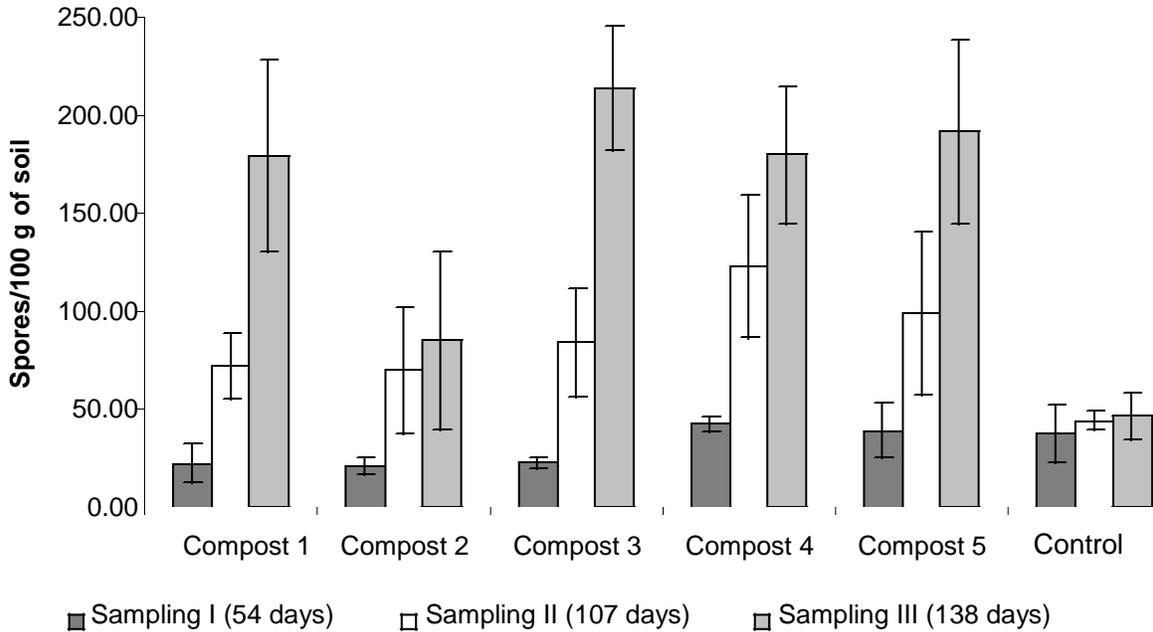


Figure 3. Population density of spores of AM fungi of plots cultivated with corn supplemented with compost and fertilized. Data are reported as the mean values range for three replicates.

with beans and broad beans growth in Tlaxcala, that the number of spores varied with the phenology of the plant, with low quantities at the time of planting, maintaining until flowering, and increases at the stage of fruit ripening (Luna-Zendejas, 1997). Cousin et al. (2003) showed that the sporulation of different species of AM fungi were clearly seasonal and influenced by host plant phenology of different ecosystems as we found. The presence of fungal structures (mycelium, arbuscules and vesicles) reach their peak during the flowering stage of corn; at that time the plant has a high demand for carbohydrates, while the populations of spores of AM fungi presented the highest values at the end of the rainy season (Gavito and Varela, 1993).

With the addition of the composts was achieved a positive effect on growth, agricultural production of corn and in soil properties. These results suggest that the sludge could be used to make compost instead to sent them to final disposition, to correct the problem of erosion of topsoil. The soil will also retains more water and improves the cation exchange capacity, which will give fertility to the same, and therefore have better yields to future crops. Application of compost also create the appropriate environment for the growth of bacteria, fungi and actinomycetes, beneficial to soil and plants, This microorganisms make symbiotic relationships with plants by provide nitrogen, solubilize phosphorus, favor the absorption of nutrients and water by plants and stimulate the growth of roots, especially in the early stages of their growth. Also create an environment hostile to plant pathogens, reducing the incidence of diseases in different plant species.

Conclusion

The use of sludge from wastewater treatment plants is an interesting way to use their nutrients for the production of compost, a material with good physical and chemical properties that allow increase in the growth and yield of corn. Also compost made with sludge could be used to reduce environmental problems such as erosion, because the continued application of could improve the soil quality and physical and chemical properties. Developing the culture of sludge recycling will be beneficial: for example in the restoration of lands, avoid contamination by fertilization and sites of final disposition will increase their middle life. All this is possible because the organic matter, regardless of its nature, could be mineralized through the composting process, resulting in a non-phytotoxic material, where components are transformed into compounds assimilated by crops.

ACKNOWLEDGMENT

We are grateful to Mr. Ascension Altamirano for allowing us to carry out the experimentation on his land of municipality of the Altzayanca, Tlaxcala, Mexico.

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