Microbial activity and fungal composition of composted animal manure suppressive to soil-borne pathogens.

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Abstract

Microbial composition in composted organic materials is an important factor to be considered when producing compost for disease suppression. The objectives of the study were to evaluate both microbial activity and fungal composition in composted animal manure with varying degree of suppressiveness towards soilborne fungal pathogens. Microbial activity of composted cattle, pig, poultry and sheep manure and loamy field soil was tested by means of hydrolysis of fluorescein diacetate (FDA). Fungal populations in these composts were also studied by serial dilution technique. Microbial activity was significantly higher (P<0.05) in poultry, pig and cattle manure compost as well as field soil compared to composted sheep manure which had the lowest microbial activity. High fungal populations were recorded in cattle (62%), poultry (63%) and pig (65%). Trichoderma harzianum, T. viride, Talaromyces trachyspermum and Penicillium verrucosum were the most common species isolated from cattle, poultry and pig manure composts. Significantly (P<0.05) less species were observed in both control (48.6%) and composted sheep manure (12.5%), with Humicola sp. being the only species isolated from this compost. Microbial composition of composted animal manure is an ideal indicator of the suppressiveness of such composts towards soilborne pathogens.

Keywords:

1. Introduction

Increasing interest in composting as a waste management strategy has led to more research efforts being directed toward the utilization of composts in agricultural crop production (Craft and Nelson, 1996). Composting of organic wastes prior to agricultural use reduces odor, decreases undesirable physical properties and increases nutrient availability (Voland and Epstein, 1994). Compost is also an excellent soil conditioner, which can provide nutrients to plants in an easily available form. Composts are known to improve the physical and chemical properties of soil, and generally enhance the activity of soil microorganisms (Hideaki et al., 1990). Besides directly enhancing plant growth, some composts are also known to suppress plant diseases (Voland and Epstein, 1994).

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The control of soilborne plant pathogens with compost is a relatively recent horticultural approach, which has been explored in by numerous researchers (Boehm and Hoitink, 1992; Hadar et al., 1992). Composts prepared from varying sources like tree bark, municipal sewerage sludges and different types of animal manure reportedly suppresses many soilborne plant pathogens including Pythium ultimum Trow, Rhizoctonia solani Kuhn and Phytophthora cinnammomi Rands (Chen and Hadar, 1986; Chen et al., 1988; Aryantha et al., 2000). Hadar and Mandelbaum (1992) described suppressive compost as an environment in which disease development is reduced despite the introduction of pathogens in the presence of susceptible plants. Disease suppression in this case, is the direct result of the activity of antagonistic microorganisms (Hadar and Mandelbaum, 1992).

The suppression of soilborne plant pathogens by composts is mainly due to increased microbial activity (Hoitink and Fahy, 1986), with a characteristically high bacterial-to-fungal ratio. Many fungal and bacterial antagonists of plant pathogens have been isolated from organic composts (Mandelbaum and Hadar, 1990) and direct relationships between microbial activity and pathogen suppression have been established (Chen et al., 1988; Mandelbaum and Hadar, 1990). Numerous species of Trichoderma and certain bacteria, including species of Enterobacter, Flavobacterium, Xanthomonas and various fluorescent Pseudomonas species have been isolated from composts suppressive to R. solani (Nelson and Craft, 1992). The combination of these organisms results in the increased suppression of R. solani in compost amended growth media.

Microbial activity in composts can be determined by examining the concentration of different groups of microorganisms e.g. aerobic bacteria, anaerobic bacteria, fungi, actinomycetes, pseudomonads and nitrogen fixing bacteria by dilution plating (Hoitink et al., 1991). However, microbial activity, based on the rate of hydrolysis of fluorescein diacetate (FDA) by microorganisms, has afforded new and useful insights into the mechanisms by which microorganisms suppress the activity of soilborne pathogens (Marull et al., 1997).

The diversity of fungi in compost can be indicative of the compost being either conducive or suppressive to soilborne plant diseases (Kuter et al., 1983). A high density of Trichoderma hamatum (Bonorden) Bainier and T. harzianum Rifai are characteristic of growth media suppressive to Rhizoctonia spp. In contrast, populations of Penicilium verrucosum Westling Samson and Geomyces spp, are high in media conducive to diseases caused by Rhizoctonia (Kuter et al., 1983).
The objectives of this study were firstly, to measure the microbial activity of four animal manure composts utilized frequently in Lesotho for seedling production and secondly, to identify and quantify species of fungi present in these composts.

2. Materials and methods

Compost and soil samples. Composts used in the study were prepared from cattle, poultry, pig and sheep manures. Compost piles were made-up of animal manure and garden waste. Mature compost were then kept in paper backs at room temperature until further use. Soil samples representing three different soil series: 1. Maseru (sandy clay with 3.95 % organic matter); 2. Sephula (sandy clay loam; 3.68 % organic matter); and 3. Leribe (clay loam; 2.34 % organic matter) were obtained from three fields previously cultivated with peas (Pisum sativum L.), cabbage (Brassica oleracea L.) and tomato (Lycopersicon esculentum Mill.). Ten samples from each soil series were passed through a 6 mm sieve and stored at room temperature until further use. A sub-sample (500 g) was air dried at 40 ^{o} C for 1 hour and used for FDA hydrolysis and fungal population assessment.

Microbial activity. Microbial activity in both composted animal manure and field soil (control treatment) was determined by measuring the rate of hydrolysis of fluorescein diacetate (FDA) (Schnurer and Rosswall, 1982). FDA was dissolved in acetone (2.0 mg/ml) and stored as a stock solution at -20 ^{o} C. Twenty milliliters of sodium phosphate buffer (pH 7.6) was added to individual soil and compost samples (0.5 g) placed in 250 ml Erlenmeyer flasks. The FDA stock solution was added (0.2 ml) to the mixture, which was then incubated for 1 hour in a rotary shaker (90 rpm) at 25 ^{o} C. Each treatment was replicated three times. A control sample not containing organic matter was used to correct background absorbance for each soil and compost sample. Compost residues were removed from the FDA-buffer mixture by filtering the suspension through filter paper (Whatman No.1). The filtrate was collected in a test tube, covered with Parafilm®, and placed in an ice bath for 30 minutes. The concentration of free fluorescein after hydrolysis was determined by reading the optical density at 490 nm using spectrophotometer after 30 minutes.

Standard curves for each compost and soil was prepared by adding various quantities of FDA (0, 100, 200, 300, 400 µl in the stock solution) to 5 ml of phosphate buffer (pH 7.6) in test tubes. Tubes were tightly closed and placed in a boiling water bath for 60 min. After cooling for 10 min, hydrolyzed fluorescein was added to 250 ml Erlenmeyer flasks containing 0.5 g (dry weight) of each compost and soil. In order to remove all remaining traces of fluorescein, test tubes were rinsed with 15 ml of phosphate buffer, which was then also poured into the flasks. The flasks were placed on a rotary shaker for 20 minutes and the reaction was stopped by adding 20 ml of acetone to all samples. Compost and soil samples were filtered through a No 1 Whatman filter paper. The filtrate was then collected into test tubes, covered with Parafilm® and placed into an ice bath to prevent acetone volatization. The concentration of free fluorescein in the filtrate was then determined as described above.

Fungal population density. Fungal populations in the four composted animal manures and three soil samples were isolated and enumerated by means of dilution plating.

Compost or soil (1 g) was placed in 99 ml of 0.1% water agar and the mixture was agitated for 45 seconds on high speed in a Waring blender. The suspension was then diluted (10^{-1}) and 0.1 ml of the final dilution was aliquoted onto 25 plates each containing water agar amended with 300µg/ml streptomycin sulphate (Novostrip). Plates were incubated at 22 ^{o}C and the total number of fungal colonies growing in each plate was recorded after 48 hours. Hyphal tips were removed from 50-70 colonies on each plate with the aid of a dissecting needle and microscope, placed onto potato dextrose agar (PDA) (Difco) plates and incubated for 7-10 days. The resulting cultures were then sorted into presumptive groups based on cultural morphology. Representative cultures of each group were given identification numbers and set aside for detailed examination and identification. Standard mycological procedures were used to identify genera which could not readily be classified to species level by means of morphological characteristics. A mean of relative frequency of fungal genera or species from each compost and soil sample was calculated (total colonies of fungal species / number of replicates). Each experiment was conducted three times and an analysis of variance (ANOVA) was performed with the combined data after conducting Bartlett's test for homogeneity of variances on fungal population densities. Means were separated using Duncan's multiple range test at the 95 % confidence level.

3. Results

Microbial activity. Significant differences in microbial activity were observed between the four composted animal manures and field soil (combined mean of three samples). Microbial activity was significantly higher ($P < 0.05$) in composted pig, poultry and cattle manure and lower in field soil and sheep manure compost (Figure 1). The highest level of microbial activity was observed in composted pig manure. Microbial activity in cattle and poultry manure was significantly lower than pig manure but was still significantly ($P < 0.05$) higher than field soil and composted sheep manure.

There was a significant difference ($P < 0.05$) in the absorbance of fluorescein between the four composted animal manures and field soil. A good calibration curve could however not be obtained from all four composts (Figure 2 A). Fluorescein absorbance was high in poultry, cattle and pig manure composts at all FDA concentrations. Soil samples had a lower rate of FDA absorbance than the above mentioned three composts (Figure 2 B). FDA absorbance in field soil was however higher than in composted sheep manure, the latter not differing significantly in this respect from the blank sample (control treatment).

Fungal populations. Various species of fungi were isolated from the four animal manure composts and three soil types (Table 2). A high number of fungal species were isolated from cattle, pig and poultry manure compost. These included Trichoderma viride Pers., Talaromyces trachyspermum, Penicillium verrucosum, Rhizopus spp,

Aspergillus repens and Humicola spp High populations of Trichoderma spp. were isolated from cattle, pig and poultry manure compost (Table 2). Sheep manure yielded the lowest number of fungal isolates (Figure 3), with Humicola sp. virtually the only species isolated from this compost. The same species also occurred in relatively high numbers from composted pig manure but was virtually absent in the other two composts and field soil. In cattle manure compost, Rhizopus oryzae Wenr & Frinsen Geerlings was the most frequently isolated fungus followed by A. repens and Trichoderma harzianum Pers. The most common fungi in composted poultry manure compost were A. repens T. harzianum and P. verrucosum.

4. Discussion

Microbial activity in composted organic wastes, measured by the rate of fluorescein diacetate hydrolysis and fungal populations, is an important criterion in determining the conduciveness or suppressiveness of composts to plant disease (Kuter et al., 1983; Inbar et al., 1991; Klamer and Søchting, 1998). In the present study, microbial activity and fungal population densities of four composted animal manures were assessed. High rates of fluorescein diacetate were absorbed in composted cattle, poultry and pig manure, which was consistent with the population density of fungi in these composts. Similarly, low population densities of fungi in sheep manure were consistent with a low rate of fluorescein diacetate hydrolysis.

The application or incorporation of organic products into soil creates a dynamic soil ecosystem by promoting microbial activity, which as a result increases soil enzyme activity (Martens et al., 1992; Press et al., 1996). Microorganisms in soil and compost produce enzymes such as protease, lipase, and esterase, which are responsible for the hydrolysis of FDA (Press et al., 1996). Spectrophotometry measurements at an optical density of 490 nm, can determine the amount of free fluorescein in compost and soil (Inbar et al., 1991). High rates of free fluorescein were observed in composted sheep manure and control soil samples, compared to a significantly lower rate in composted cattle, poultry and pig manure. Since various enzymes in living cells are responsible for FDA hydrolysis, high rates of free fluorescein in sheep manure is an indication of low microbial activity. Low rates of free fluorescein in composted cattle, poultry and pig manure thus indicates a higher presence of microorganisms in these composts.

By comparing the fungal species isolated from the various composts and field soil, certain trends regarding the distribution of fungi in these substrates were evident. For example, Humicola sp. which was more dominant in sheep manure compost, has been shown to withstand unfavourable conditions such as high temperatures and toxicity (Straatsman et al., 1994, Aryantha et al., 2000). Sheep manure compost had high levels of zinc and phosphorus and low pH levels (Table 1), which might have promoted colonization of sheep manure compost by this fungus.

Presence of Trichoderma species in compost is considered as an indication of high suppressiveness of such compost towards soilborne pathogens (Hoitink and Fahy, 1986). In our pervious studies (unpublished data), these composts were highly suppressive towards virulent isolates of R. solani, P. ultimum and F. oxysporum. Aspergillus spp. has also been isolated from highly suppressive compost (Hoitink and Fahy, 1986). The same trend was observed in our study where Aspergillus spp. was frequently isolated from cattle and poultry manure compost, which also had a high degree of suppressiveness towards soilborne fungal pathogens.
Numerous microorganisms found in composted growth media act as antagonists of soilborne plant pathogens (Boehm et al., 1993; Press et al., 1996). The ability of compost to suppress pathogenic organisms is attributed to the fact that microflora in compost is able to prevent spore germination of pathogens due to competition for nutrients, lysis or hyperparatism (Hadar et al., 1992). Composted animal manure used in this study had a significant amount of organic nutrients (Table 1), which can in turn result in increased microbial activity.

Results obtained in this and other previous studies clearly indicate that microbial activity and fungal populations in composted manure can determine the ability of such composts to suppress soilborne plant pathogens. The low microbial activity observed in composted sheep manure renders this unsuitable for disease suppression. Studying the association between high microbial activity, fungal populations and disease in compost amended soil media is the first step in understanding the concept of disease suppression by the four, composted animal manure evaluated in this study. A better understanding of the dynamics involved in the suppressive effects of composted animal manure used by farmers in Lesotho will hopefully provide more effective and economical production methods in Lesotho. This can also provide alternative control strategies which are safe and affordable for sustainable management of soilborne diseases in Lesotho.

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Table 1: Chemical composition of poultry, cattle, pig and sheep manure compost used as seedling production in Lesotho.

	Types of compost			
Chemical component	Poultry	Cattle	Pig	Sheep
pH	7.84	8.02	7.82	5.47
P (%)	1.83	1.6	1.45	2.47
N (%)	0.14	1.68	1.32	2.33
Ca (%)	0.35	0.31	0.32	1.37
Mg (%)	0.81	0.74	0.68	0.47
K (%)	0.09	0.08	0.07	0.17
Na (%)	1.58	1.55	1.18	1.33
Org C (%)	9.18	7.6	13.19	9.83
Zn (dpm)	525	425	450	925
Fe (dpm)	8100	7600	7250	4500
Mn (dpm)	425	387	435	200
Cu (dpm)	110	75	125	27

Table 2: Fungal species isolated from composted animal manure and field soil

	Populations in compost (%)				
Fungal species	Cattle	Poultry	Pig	Sheep	Soil
Aspergillus repens	33.2	32.8	3.6	0.2	30.2
Penicillium purpurogenum	35.8	25.4	3.2	0	35.7

Torulamyces lagana Delitsch	32.3	43.4	7.7	0.1	16.4
Penicillium griseovulvum Dierctx	42.7	12.9	7.2	0.7	36.5
Trichoderma viride Pers ex Gray aggr.	23.2	21.3	32.4	1.2	21.9
Paecilomyces inflatus Burnside Carmichael	12.2	22.9	35.6	5	24.2
Geotrichum sp	17.7	34.4	35.3	7.2	5.4
Penicillium citrichum Thom	4.8	27.4	45.2	0.6	22
Rhizopus oryzae Went & Prinsen Geerlings	36.9	18.8	7.7	3.2	33.5
Humicola sp	4.2	2.9	52.3	34.1	6.3
Fusarium oxysporum	12.7	8	4.2	0	75.1
Talaromyces trachyspermum	25.7	19.8	31.7	3.4	19.3
Sphaeronaemella fimicola	35.8	20.9	14.4	1.4	27.6
Aspergilus fumigatus Fresen	21.2	35.6	29.6	2.4	11.2
Penicillium verrucosum Westling Samson.	23.9	25.2	32.7	1.1	16.9
Trichoderma harzianum Rifai	30.5	28.5	29.3	0.1	11.6
Penicillium ochrochloron Biourge	13.3	27.8	25.4	1.7	31.8
Unidentified species	19.3	9.6	27.7	6.6	36.8

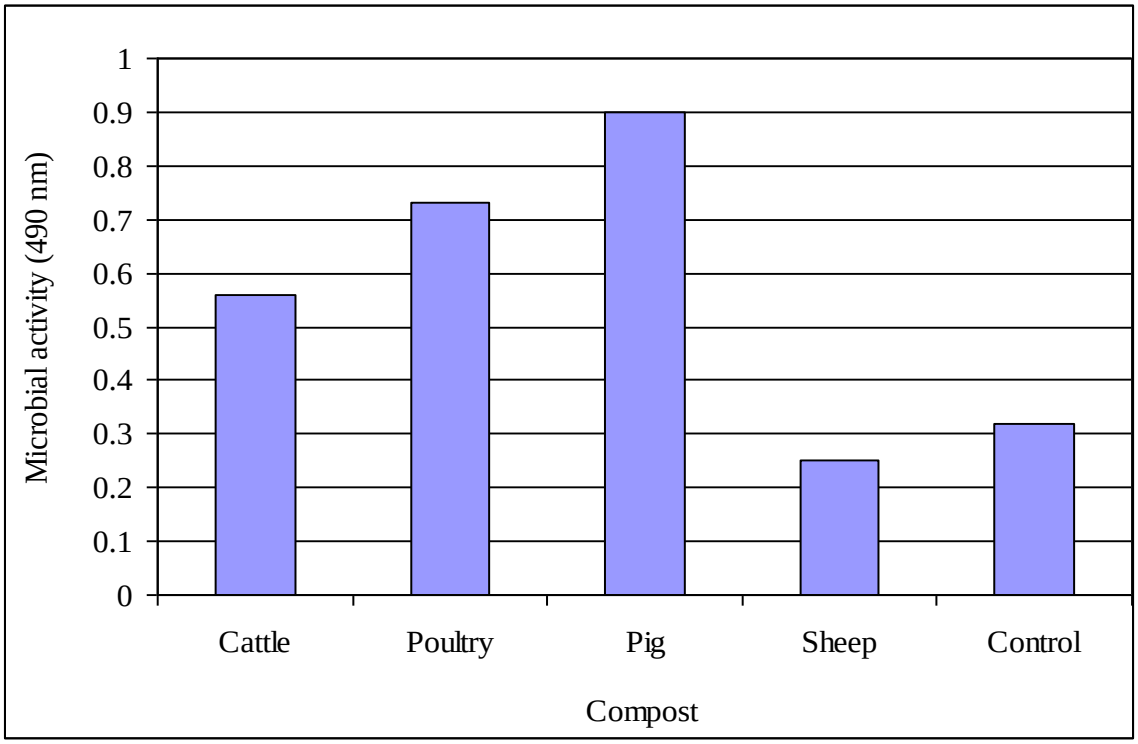

Figure 1: Microbial activity in composted animal manure and soil as measured by FDA hydrolysis. Control treatment contained neither soil nor compost.

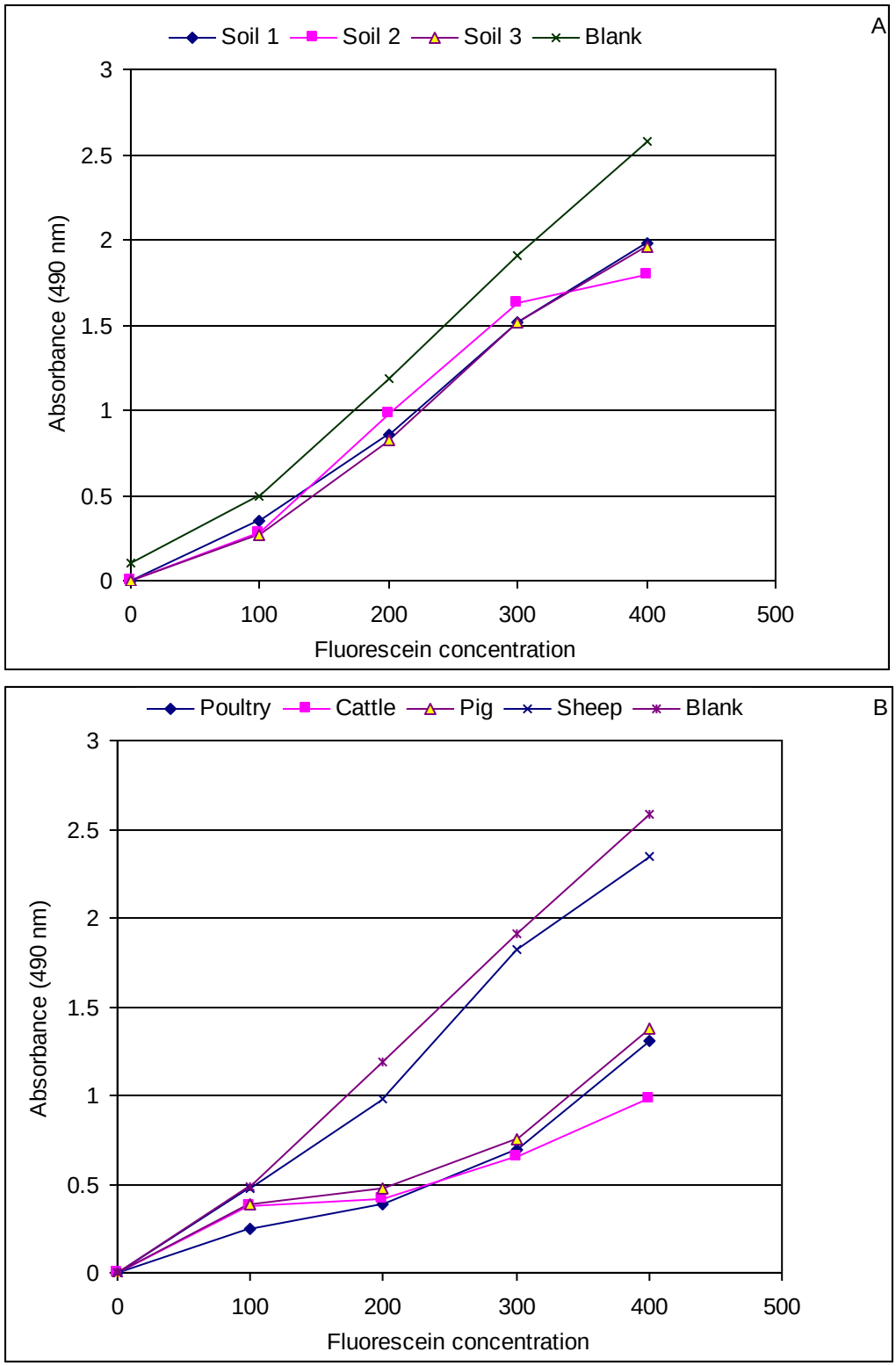

Figure 2: Relationship between hydrlysed FDA concentration and optical density (490 nm) for soil (A), and composted animal manure (B). Bland sample contained neither soil nor compost.

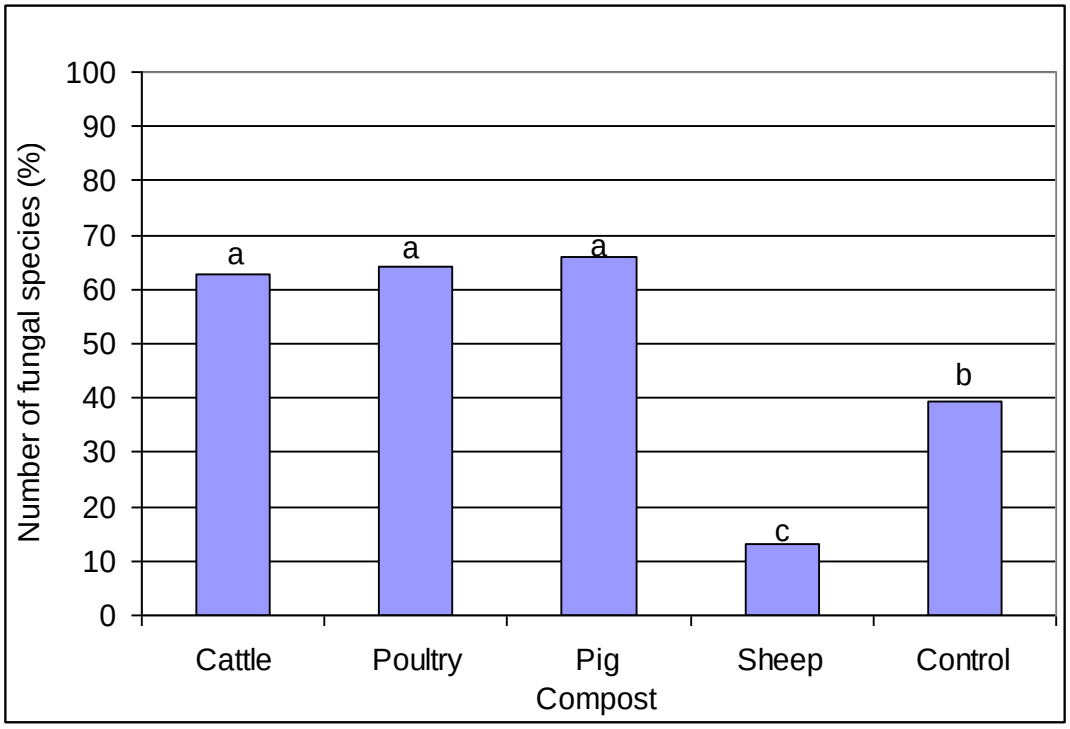

Figure3: Population densities of fungal species isolated from different composted animal manure and field soil. Each datum point is a mean of eight replications per treatment. Bars denoted by the same letter are not significantly different according to Duncan's multiple range test (P>0.05).