

Microbial Diversity during Composting Cycles of Rice Straw

Mohamed Hefnawy¹, Osama M. Nagdi²

¹Botany department, Faculty of Science Menoufia University, Egypt

²National Agriculture research Center, Giza, Egypt

Abstract: *Mesophilic bacteria, fungi and actinomycetes varied during composting cycles with high numbers in the initial and final cycles with maximal values in compost C and D, and sharply decreased in the heating cycles. Staphylococcus aureus and Bacillus subtilis were dominated at the initial composting cycle. Whereas, at maturity Bacillus subtilis was the major followed by B.badies, B.polymixa and B.brevius and exhibited high numbers in compost C and D. Thermophilic B.stearothermophilus, Thermus sp. and other Bacillus sp. were the major in the heating cycles (20,40 days) with maximum values in compost A and D. Fusarium oxysporium and F. moniliforme disappeared at the heating cycles while, Rhizopus nigricans was the major mesophilic fungus found in compost heaps with maximum value in compost D. Aspergillus fumigatus was dominated in the heating cycles with high frequency also in compost D. Trichoderma viride and T. reesei appeared only in cooling cycle and dominated in compost D. Streptomyces antibioticus, St. cinnaborinus, St. roses, Thermo dichotomicus and Thermo vulgaris exhibited high frequencies in all compost heaps in initial and cooling cycles. While, Thermo dichotomicus and Thermo vulgaris were dominated in heating cycles. Microbial succession and community dynamics started by high numbers of mesophilic bacteria, actinomycetes and fungi in the initial phase followed by high numbers of thermophilic ones in the heating phase whereas, other mesophilic organisms appeared in the final cooling phase. Mixing equal ratios of Rice, wheat, clover, faba bean and maize straw (compost D) might be more suitable in composting of rice straw.*

Keywords: Composting, fungi, bacteria, actinomycetes, physical and chemical parameters.

1. Introduction

Composting is the biological conversion of solid organic waste into usable end products such as fertilizers, substrates for mushroom production and biogas. Moreover, their high organic matter content and biological activity make composts effective in a variety of applications, including erosion control, revegetation, biofiltration and bioremediation [1].

Increasing human populations needs a new agriculture land by adding newly reclaimed sand soils which are poor in its organic matter content. Maintaining and enhancing the fertility and productivity of agricultural soil have had a high priority in the last decades [2]. The most important method to improve soil quality involves recycling of crop residues [3]. Composting is a microbial process that converts organic waste into a nutrient-rich end product used in horticultural and agricultural applications. Therefore, the recent trends in recycling of crop residues indicate the possibility of using certain efficient microflora particularly the best known cellulose and lignin degraders. If the microflora were added during composting the time of composting might be reduced [4].

Mesophilic composting is more effective for mass reduction in rice straw cattle manure compost than thermophilic composting because of the higher decomposition activity of the microbial community characterized by the predominance of protobacteria and fungi [5].

During composting of different wastes or residues it is obvious that a high microbial activity with a succession of microbial populations depending on the temperature reached during the biodegradation processes [6], [7], [8].

Four typical phases of composting were observed during composting of five piles (1.5 x 1.0 x 0.80 m³) containing mainly rice straw, soybean residue and enriched with rock phosphate: short initial mesophilic phase followed by, thermophilic, cooling and maturation phases. Physicochemical changes confirmed the succession of microbial populations depending on the temperature of each phase in all treatments. Intense microbial activities led to organic matter mineralization and simultaneously narrow C/N ratios [9]. This work aims to investigate the microbial succession and dominance in four compost heaps during composting phases.

2. Materials and Methods

Preparation of compost heaps:

Four compost heaps were prepared from plant residues of rice, wheat, faba bean, maize and clover straw with equal ratios. The heaps (1x1x0.7m) were set up as in the following:

- A. Rice, wheat, faba bean and maize straw
- B. Rice, wheat, clover and maize straw
- C. Rice, faba bean, clover and maize straw
- D. Rice, wheat, clover, faba bean and maize straw

Each type of heap was inoculated with 1kg/ton with compost starter to initiate composting processes. The moisture of the heaps was adjusted to approximately 70%, the compost heaps were also turned up every 10 days.

Microbial investigation in compost heaps:

Three different compost samples were taken in sterile polyethylene bags from each heap at depth of

approximately 30cm. The samples were collected at 0, 3, 20, 40, 80 days of composting periods. 5 gm from each compost sample was placed into a bottle containing 45 ml sterile distilled water, shaken well for 10 min and serial dilutions were prepared.

The total number of bacteria, fungi and actinomycetes were determined by inoculating 0.5 ml of suitable dilution onto agar plates with specific medium, nutrient agar, potato dextrose agar and starch ammonium agar respectively. Five plates were prepared as a replicates for each compost sample, one set of plates were incubated at 30°C, while other group was incubated at 55°C. The total microbial number was counted.

Purification and identification of more common microbes:

The most common fungal colonies were sub-cultured on Dox agar medium for further purification and were identified according to [10], [11], [12]. The identified fungal species were compared and confirmed by the culture collection of Botany Department, Faculty of Science, Cairo University, Egypt. Nutrient agar medium was used for purification and identification of Bacteria. While, Jensen's medium for actinomycetes [13]. Bacterial species were identified by Biotechnology and Applied microbiology Center, Al-Azhar University, Cairo, Egypt. Whereas, Actinomycetes isolates were identified in Microbiology department, National Research Center, Giza, Egypt.

3. Results

Temperature changes of compost piles: The temperature of the compost heaps at different depths (0, 30, 50cm) showed a slight increase at the initial time with various depths. Whereas, at 20 days of composting the temperature of the heaps showed a marked increase at a depth of 30 and 50cm reached to approximately 57o and 69° C respectively, and exhibited the maximum recorded temperatures during the composting process. While, at 40 and 60 days of composting the temperatures of the heaps showed a marked decrease till it reached to the original temperature at the beginning of the composting process (Fig.1).

Total microbial count during composting cycles:

At the beginning of the composting cycles the total number of mesophilic bacteria, fungi and actinomycetes was high while, it sharply decreased in the heating phase at 20, 40 days comparing with the first cooling cycle. Whereas, they markedly increased again in the second cooling phase at 80 days and its number was higher than in the first cycle.

In contrast to the above results, thermophilic bacteria, fungi and actinomycetes exhibited very low number in the first and second cooling cycle of composting while, they showed a sharp increase in their numbers at 20 days of composting. These results were also observed with all tested compost heaps. The best number of these

microorganisms was found in compost D comparing with other compost A, B and C (Table 1-6).

Bacterial colonization of compost heaps:

The most common identified bacteria which encountered the compost piles at the first and final stages of composting cycles and represented the mesophilic bacteria were *Staphylococcus aureus*, *Staphylococcus xylozeus*, *Bacillus sp.* *B. subtilis*, *B. brevis*, *B. polymyxa* and *Klebsiella pneumonia*. *Staphylococcus sp.* was the dominant species at the beginning of composting process in all compost types. Whereas, it completely disappeared after 20 days. On the other hand, other *Bacillus sp.* was dominated during the reminder of the composting periods. The frequency of *Bacillus subtilis* in all compost heaps ranged from 26.7% to 56.1% of the total bacterial count. *Klebsiella pneumonia* appeared only at 80 days. *B. subtilis*, *B. brevis* and *B. badis* were found in high numbers in compost C and D at 40 and 80 days of composting (Table 1).

In contrast, during the thermophilic cycle the pathogenic bacteria disappeared particularly after 3 weeks of composting process. *Bacillus stearothermophilus* exhibited the most dominant bacterial species in heating phase until the end of composting cycles, its frequency ranged from 36.8 up to 83.4% with all compost heaps, their maximum existence was found in compost D whereas, the maximum existence of thermo sp. was observed in compost B (Table 2).

Fungal colonization of compost heaps:

The mesophilic fungi which colonized the compost heaps were *Aspergillus niger*, *Fusarium oxysporum*, *Fusarium moniliforme*, *Rhizopus nigricans* and *penicillium citrinum*. At the beginning of composting cycle, *Rhizopus nigricans* exhibited the highest frequency in all compost heaps and ranged from 25 to 50%, its maximum existence was found in compost D

It was also observed that pathogenic fungi, *Fusarium oxysporum* and *Fusarium moniliform* disappeared after 20 or 40 days of composting time, other fungal species such as *Trichoderma viride* and *Trichoderma reesei* exhibited high frequency at the end of composting cycles (Table 3).

The most dominant thermophilic fungal species were recorded at 20 and 40 days of composting periods were *Aspergillus fumigatus* and *Aspergillus fumigatus var elipticus* with high frequency, their maximum existence was also found in compost D (Table 4).

Actinomycetes colonization of composting heaps:

Five mesophilic *Streptomyces sp.*, *Streptomyces antibioticus*, *S. cinnaborinus*, *S. roseus*, *S. griseus* and *S. aureofaciens* were found in all compost heaps and they were exist during all compost cycles. *St. antibioticus*, *St. cinnaborinus* and *St. roses* exhibited high frequencies with all compost heaps from the beginning phase till the end

periods of composting 80 days. *S. cinnaborinus*, *S. griseus* and *S. aureofaciens* exhibited the best numbers in compost D at 80 days. On the other hand, a few numbers of thermophilic actinomycetes were exist during the composting periods from the beginning till the end cycle. *Thermo dichotomicus* and *Thermo vulgaris* were the major actinomycetes which exhibited high frequency with all compost heaps especially in compost D (Table 5, 6)

4. Discussion

Composting is one of the most promising avenue for recycling plant residues and used it as organic manures to improve its quality and fertility. During composting processes, the temperature evaluation reflect the microbial activity and determine the composting efficiency [14], [15]. In this study the temperature of all tested compost heaps was highly elevated after the third day of composting and exhibited the maximum level (55°C and 68°C) at 20 days at a depth of 30 and 50 cm of the heap respectively. The results refer to high microbial activity at this time of composting which is more suitable to kill most pathogenic microbes. The obtained results are similar to that obtained by [16] they also mentioned that rising the compost temperature up to 65°C was desirable to kill most pathogens.

In the present study, the number of mesophilic bacteria, fungi and actinomycetes was high in the early and final phase of composting in all compost heaps especially in compost D, this was also observed with thermophilic ones in the heating cycles. Mesophilic bacterial species exhibited variation in their number and existence at different compost periods and compost types. *Staphylococcus aureus*, *Staphylococcus xyloesus*, *Staphylococcus sp.* were the dominant species at the beginning of composting process in all compost types. Whereas, it completely disappeared after 20 days. *Bacillus sp.* *B. subtilis*, *B. brevis*, *B. polymyxa* started the first compost cycle with low count and their members were increased with the advancement of the cycle especially in compost C and D. Thermophilic, *B. stearothermophilus* dominated in the heating cycles with maximum existence in compost D. This was also reported by [17], [18] they also reported that mesophilic *Bacilli* are considered to be primary decomposers, while, thermophilic ones are secondary decomposers. The number of *Staphylococci* were increased at the beginning of composting cycle while, they completely disappeared at the fourth week due to rising of temperature, which sterilize the compost against these pathogens.

The most mesophilic fungi which dominate in the initial stage of composting were the genera *Aspergillus*, *Fusarium*, *Penicillium* and *Rhizopus*. The pathogenic *Fusarium sp.* disappeared completely in the heating phase while, *Trichoderma reesei* and *T. viride* appeared at the final cooling cycle. *Rhizopus nigricans* dominated in all compost heaps with high number in compost D. Thermophilic, *Aspergillus fumigates* was found in the heating cycles especially in compost D. [19] stated that, at the beginning till 15 days of composting the thermotolerant fungi such as *Cladosporium*, *Aspergillus*,

Mucor, *Rhizopus* and *Absidia spp* were isolated. The thermophilic fungi which isolated from the tested compost during the heating phase were *Aspergillus fumigates*, *Aspergillus spp.*, *Humicola spp.*, *Talaromyces spp.* and *Thermomyces spp.* Similar results were obtained by [18] they isolated *Aspergillus fumigates*, *Humicola spp.*, and *Talaromyces spp.* during composting of de-inking paper sludge. In this study, number of *Streptomyces spp* were found in all compost heaps and they were exist during all composting cycles. *Streptomyces antibioticus*, *St. cinnaborinus* and *St. roses* exhibited high frequencies with all compost heaps from the beginning phase till the end periods of composting (80 days). As well as, thermophilic actinomycetes were also exist. *Thermo dichotomicus* and *Thermo vulgaris* were the major actinomycetes which exhibited high frequency with all compost heaps with high numbers in compost D. The presence of actinomycetes during composting process exhibited an enzymatic activity to decompose resistant carbon fractions [18]. Quite similar results revealed that succession of communities of different bacteria and fungi mainly was observed during composting of chicken feathers with pine bark (FB) and with pine bark/rye straw (FBS). The succession was dominated by fungal than bacterial communities. Bacteria, including Actinomycetes, grew intensively during the first 2-4 weeks of composting and included mainly proteolytic, rarely cellulolytic, populations; afterwards, bacteria were gradually replaced by fungi [8].

In conclusion: Microbial community highly changed during composting cycles from initial, heating and final cooling cycle. The number and existence of microorganisms showed high variation between mesophilic and thermophilic bacteria, fungi and actinomycetes. High numbers of these microorganisms were found in compost D at all composting cycles and exhibited the best mixtures of plant residues for composting rice straw.

References

- [1] Alexander R. (1999): Compost markets grow with environmental applications. BioCycle Magazine, March:43-44.
- [2] Crecchio C., Maddalena C., Rosaria M., Patrizia R. and Pacifico R. (2004): Short term effects of municipal solid waste compost amendments on soil carbon and nitrogen content on some enzyme activities and genetic diversity. Biol. Fertil. Soil, 34: 311-318.
- [3] Lalonde R., Gagnon B., Simard R. R. and Tote D. (2000): Soil microbial biomass and enzyme activity following liquid hog manure in a long term field trial. Can. J. Soil Sci., 78: 581-587.
- [4] Buswell JA, Change and Shu- Ting (1994): Biomass and extracellular hydrolytic enzyme production by six mushroom species grown on soy bean waste. Biotechnol. Let. 16 (12):1317-1322.
- [5] Tang J.C., Shibata A, Zhou Q, Katayama A. (2007): Effect of temperature on reaction rate and microbial community in composting of cattle manure with rice straw. J. Biosci Bio eng. 104(4):321-8.

- [6] Hachicha S, Sellami F, Cegarra J, Hachicha R, Drira N, Medhioub K, Ammar E. (2009): Biological activity during co-composting of sludge issued from the OMW evaporation ponds with poultry manure- Physico-chemical characterization of the processed organic matter. *J Hazard Mater.* 162(1):402-9.
- [7] Novinscake A., De Coste NJ., Surette C., Filion M.(2009): Characterization of bacterial and fungal communities in composted biosolids over a 2 year period using denaturing gradient gel electrophoresis. *Can J Microbiol.* 55(4):375-87.
- [8] Kornilowicz-Kowalska T, Bohacz J.(2010): Dynamics of growth and succession of bacterial and fungal communities during composting of feather waste. *Bioresour Technol.* 101(4):1268-76.
- [9] Rashad FM, Saleh WD, Moselhy MA. (2010): Bioconversion of rice straw and certain agro-industrial wastes to amendments for organic farming systems: 1. Composting, quality, stability and maturity indices. *Bioresour Technol.* 101(15):5952-60
- [10] Gilman J. C. (1957): A manual of soil fungi. The Maple Press Co. New York.
- [11] Raper K. P. and Fennel D. I. (1963): The genus *Aspergillus*. Williams and Wilkems Co.
- [12] Raper K. P. and Fennel D. I. (1977): *Aspergillus*. Robert company, Huntington New York.
- [13] Allen O. N. (1950): Experimental in soil bacteriology. Burgess publishing Co., USA.
- [14] Miller F. C. (1992): Biodegradation of solid wastes by composting. In: Martin A. M. (ed.) Biological degradation of wastes, pp. 1-30, Elsevier Applied Science, London.
- [15] Namkoony W. and Hwang E. Y. (1997): Operational parameters for composting night. *Compost Sci* 5 (4): 46-51.
- [16] Saidi N., Cherif M., Jedidi N., Mahrouk M., Fumio M., Boudabous A. and Hassen A. (2008): Evolution of biochemical parameters during composting of various wastes compost. *American J. of environ. Sci.* 4(4): 332-341.
- [17] Strom P. F. (1985): Identification of thermophilic bacteria in soil waste composting. *Appl. Environ. Microbiol.* 50: 906-913.
- [18] Charest M. H., Antounb A. B. H. and Beauchampa C. J. (2004): Dynamics of water-soluble carbon substabces and microbial populations during the composting of de-inking paper sludge. *Bioresource Technol.*, 91: 53-67.
- [19] Ghazifard A., Kermanshahi K. R. and Far Z. E. (2001): Identification of thermophilic and mesophilic bacteria and fungi in Esfahan (Iran) municipal solid waste compost. *Waste Manag. Res.*, 19(3): 257-261.

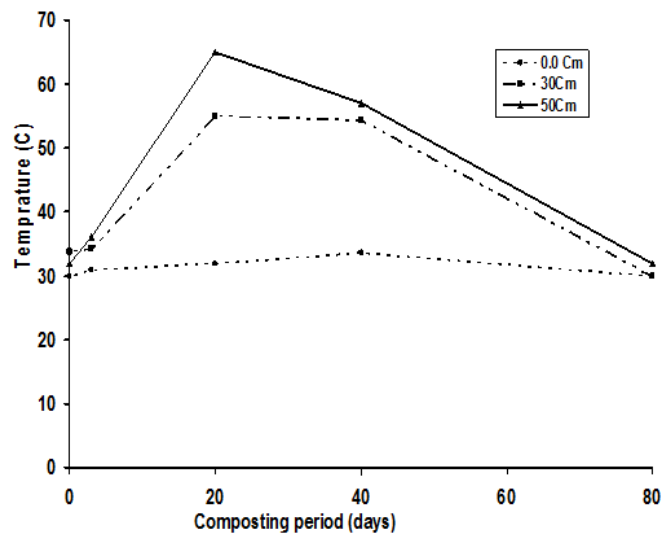


Figure 1: Temperature changes of compost heaps at different time intervals during composting process

Table 1: Colonization of mesophilic bacteria to different compost heaps at different composting periods. Data are expressed as the mean number of colony / agar plate of five replicates.

Comp. periods (days)	Bacterial sp.	Comp. A			Comp. B			Comp. C			Comp. D		
		No. of colony	Total	Freq. %	No. of colony	Total	Freq. %	No. of colony	Total	Freq. %	No. of colony	Total	Freq. %
0	<i>Staphylococcus aureus</i>	70	150	46.7	50	150	33.3	70	165	42.4	50	160	31.25
	<i>Staph. Xyloseus</i>	60		40	60		40	70		42.4	70		43.75
	<i>Bacillus sp.</i>	20		13.3	40		26.7	25		15.2	40		25
3	<i>Staphylococcus aureus</i>	50	180	27.8	60	200	30	50	200	25	80	210	38.1
	<i>Staphylococcus xyloseu</i>	0.0		0.0	60		30	60		30	70		33.3
	<i>Bacillus subtilis</i>	70		38.9	0.0		0.0	0.0		0.0	0.0		0.0
	<i>Bacillus sp.</i>	60		33.3	80		40	90		45	60		28.6
20	<i>Staphylococcus xyloseu</i>	0.0	100	0.0	5	105	4.8	0.0	100	0.0	0.0	110	0.0
	<i>Bacillus subtilis</i>	40		40	60		57.2	35		35	40		36.36
	<i>Bacillus badius</i>	40		40	0.0		0.0	0.0		0.0	30		27.27
	<i>Bacillus brevis</i>	0.0		0.0	40		38	35		35	20		18.18
	<i>Bacillus sp.</i>	20		20	0.0		0.0	30		30	20		18.18
40	<i>Bacillus subtilis</i>	50	130	38.5	55	135	40.7	40	150	26.66	60	160	37.5
	<i>Bacillus badius</i>	40		30.7	50		37	40		26.66	50		31.25
	<i>Bacillus brevis</i>	20		15.4	20		17	40		26.66	35		21.87
	<i>Bacillus polymyxa</i>	20		15.4	10		7	30		20	0.0		0.0
	<i>Bacillus sp.</i>	0.0		0.0	0.0		0.0	0.0		0.0	15		9.37
80	<i>Bacillus subtilis</i>	70	250	28	80	260	30.8	90	300	30	80	300	26.7
	<i>Bacillus badius</i>	50		20	80		30.8	80		26.7	80		26.7
	<i>Bacillus brevis</i>	40		16	50		19.2	60		20	70		23.3
	<i>Bacillus polymyxa</i>	50		20	10		3.8	40		13.3	30		10
	<i>Bacillus sp.</i>	0.0		0.0	0.0		0.0	0.0		0.0	10		3.3
	<i>Klebsiella pneumoniae</i>	40		16	40		15.4	30		10	30		10

Table 2: Colonization of thermophilic bacteria in different compost heaps at different composting periods. Data are expressed as the mean number of colony / agar plate of five replicates.

Comp. periods (days)	Bacterial sp.	Comp. A			Comp. B			Comp. C			Comp. D		
		No.of colony	Total	Freq. %	No.of colony	Total	Freq. %	No.of colony	Total	Freq. %	No.of colony	Total	Freq. %
0	<i>Bacillus sp.</i>	50	85	58.8	87	87	100	80	80	100	90	90	100
	<i>Thermus sp.</i>	35		41.2	0.0		0.0	0.0		0.0	0.0		0.0
3	<i>B. stearothermophilus</i>	50	120	41.7	70	140	50	75	147	51	80	150	53.3
	<i>Pseudomonas sp.</i>	30		25	0.0		0.0	0.0		0.0	0.0		0.0
	<i>Bacillus sp.</i>	40		33.3	70		50	72		49	70		46.7
20	<i>B. stearothermophilus</i>	70	240	29.2	80	200	40	80	210	38.1	90	230	39.1
	<i>Thermus sp.</i>	50		20.8	60		30	70		33.3	70		30.4
	<i>Bacillus sp.</i>	40		16.7	50		25	50		23.8	40		17.4
	<i>Pseudomonas sp.</i>	80		33.3	10		5	10		4.8	30		13.1
40	<i>B. stearothermophilus</i>	100	150	66.7	80	140	57.1	70	150	46.7	70	150	40.7
	<i>Thermus sp.</i>	50		33.3	60		24.9	40		26.7	50		33.3
	<i>Bacillus sp.</i>	0.0		0.0	0.0		0.0	40		26.6	30		20
80	<i>B. stearothermophilus</i>	50	60	82.4	40	75	53.3	25	30	83.4	28	35	80
	<i>Thermus sp.</i>	10		16.6	35		46.7	5		16.6	7		20

Table 3: Colonization of mesophilic fungi in different compost heaps at different composting periods. Data are expressed as the mean number of colony / agar plate of five replicates.

Comp. periods (days)	Bacterial sp.	Comp. A			Comp. B			Comp. C			Comp. D		
		No.of colony	Total	Freq. %	No.of colony	Total	Freq. %	No.of colony	Total	Freq. %	No.of colony	Total	Freq. %
0	<i>Fusarium moniliforme</i>	3	20	15	4	25	16	3	25	12	3	25	12
	<i>Aspergillus niger</i>	3		15	2		8	2		8	3		12
	<i>Fusarium oxysporum</i>	3		15	2		8	2		8	2		8
	<i>Rhizopus nigricans</i>	5		25	7		28	10		40	12		32
	<i>Aspergillus sp.</i>	3		15	3		12	4		16	3		12
	<i>Fusarium sp.</i>	2		10	2		8	3		12	1		4
	<i>Penicillium citrinum</i>	1		5	5		20	1		4	1		4
3	<i>Rhizopus nigricans</i>	7	17	41.2	10	20	50	9	21	38.1	10	20	50
	<i>Aspergillus niger</i>	7		41.2	4		20	8		42.3	5		25
	<i>Fusarium sp.</i>	1		5.9	0		0	0		0	4		20
	<i>Penicillium citrinum</i>	1		5.9	4		20	3		14.3	0		0
	<i>Aspergillus flavus</i>	0		0	0		0	2		9.5	0		0
	<i>Fusarium oxysporum</i>	1		5.9	2		10	0		0	1		4
20	<i>Rhizopus nigricans</i>	5	13	38.5	7	15	46.7	8	17	47.1	8	15	53.3
	<i>Aspergillus flavus</i>	4		30.8	6		40	3		17.6	0		0
	<i>Aspergillus niger</i>	0		0	0		0	6		35.4	3		20
	<i>Fusarium sp.</i>	4		30.7	0		0	0		0	0		0
	<i>Penicillium sp.</i>	0		0	2		13.3	0		0	4		26.7
40	<i>Rhizopus nigricans</i>	7	18	38.9	10	22	45.5	4	20	20	8	24	33
	<i>Penicillium citrinum</i>	5		27.8	6		27.3	6		30	6		25
	<i>Trichoderma reesei</i>	6		33.3	6		27.3	8		40	10		41
	<i>Aspergillus flavus</i>	0		0	0		0	2		10	0		0
80	<i>Rhizopus nigricans</i>	7	30	23.3	6	33	18.2	8	33	24.2	9	32	28.1
	<i>Aspergillus flavus</i>	5		16.7	5		15.2	0		0	1		3.2
	<i>Penicillium citrinum</i>	6		20	0		0	0		0	2		6.3
	<i>Trichoderma reesei</i>	6		20	8		24.3	10		30.3	10		31.3
	<i>Trichoderma viride</i>	6		20	8		24.2	8		24.2	10		31.3
	<i>Penicillium sp.</i>	0		0	6		18.2	5		15.2	0		0

Table 4: Colonization of thermophilic fungi in different compost heaps at different composting periods. Data are expressed as the mean number of colony /agar plate of five replicates.

Comp. periods (days)	Bacterial sp.	Comp. A			Comp. B			Comp. C			Comp. D		
		No.of colony	Total	Freq. %	No.of colony	Total	Freq. %	No.of colony	Total	Freq. %	No.of colony	Total	Freq. %
0	<i>Aspergillus sp.</i>	2	5	40	2	6	33.3	3	7	42.9	1	9	11.2
	<i>Thermomyces sp.</i>	0.0		0.0	0.0		0.0	4		59.1	4		44.4
	<i>Talaromyces sp.</i>	0.0		0.0	4		66.7	0.0		0.0	4		44.4
	<i>Humicola sp.</i>	3		60	0.0		0.0	0.0		0.0	0.0		0.0
3	<i>Aspergillus sp.</i>	3	12	25	0.0	15	0.0	0.0	15	0.0	0.0	15	0.0
	<i>Aspergillus fumigatus.</i>	6		50	5		33.3	10		67	8		53.33
	<i>Talaromyces sp.</i>	3		25	5		33.3	5		33	4		26.6
	<i>Humicola sp.</i>	0.0		0.0	5		33.4	0.0		0.0	0.0		0.0
	<i>Thermomyces sp.</i>	0.0		0.0	0.0		0.0	0.0		0.0	3		20
20	<i>Aspergillus fumigatus.</i>	10	20	50	8	23	34.8	10	25	40	11	27	40.8
	<i>Talaromyces sp.</i>	1		5	0.0		0.0	0.0		0.0	0.0		0.0
	<i>A. fumigatus var.elpticus</i>	7		35	8		34.8	10		40	11		40.8
	<i>Thermomyces sp.</i>	2		10	3		13.8	0.0		0.0	0.0		0.0
	<i>Talaromyces thermophilus</i>	0.0		0.0	4		17.4	5		20	5		18.4
40	<i>Aspergillus fumigatus</i>	7	10	70	7	11	63.3	10	100	46.7	9	12	78
	<i>A. fumigatus var.elpticus</i>	3		30	4		36.7	0.0	0.0	26.7	0.0		0.0
	<i>Talaromyces thermophilus</i>	0.0		0.0	0.0		0.0	0.0	0.0	26.6	3		22
80	<i>Aspergillus fumigatus</i>	1	1	100	1	1	100	3	3	100	4	4	100

Table 5: Colonization of mesophilic actinomycetes of different compost heaps at different composting periods. Data are expressed as the mean number of colony / agar plate of five replicates.

Comp. periods (days)	Bacterial sp.	Comp. A			Comp. B			Comp. C			Comp. D		
		No.of colony	Total	Freq. %	No.of colony	Total	Freq. %	No.of colony	Total	Freq. %	No.of colony	Total	Freq. %
0	<i>Streptomyces antibioticus</i>	15	50	30	18	52	34.6	20	55	36.4	22	58	37.9
	<i>Streptomyces cinnaborinus</i>	15		30	12		23.1	0.0		0.0	0.0		0.0
	<i>Streptomyces roseus</i>	10		20	12		23.1	13		23.6	18		31.1
	<i>Streptomyces griseus</i>	10		20	10		19.2	10		21.8	0.0		0.0
	<i>Streptomyces sp.</i>	0.0		0.0	0.0		0.0	12		18.2	18		31
3	<i>Streptomyces antibioticus</i>	25	60	41.7	30	70	42.8	32	70	45.7	25	85	24.4
	<i>Streptomyces roseus</i>	25		41.7	20		28.6	22		31.4	22		25.8
	<i>Streptomyces cinnaborinus</i>	10		16.6	20		28.6	16		22.9	18		21.1
	<i>Streptomyces griseus</i>	0.0		0.0	0.0		0.0	0.0		0.0	20		23.6
20	<i>Streptomyces antibioticus</i>	8	22	36.4	15	30	50	17	32	53.1	18	33	54.5
	<i>Streptomyces roseus</i>	7		31.8	10		33.3	10		31.3	12		36.4
	<i>Streptomyces cinnaborinus</i>	7		31.8	5		16.7	5		15.6	0.0		0.0
	<i>Streptomyces sp.</i>	0.0		0.0	0.0		0.0	0.0		0.0	3		9.1
40	<i>Streptomyces antibioticus</i>	20	65	30.7	25	65	38.5	30	70	42.9	25	75	33.3
	<i>Streptomyces aureofciens</i>	15		23.1	0.0		0.0	0.0		0.0	0.0		0.0
	<i>Streptomyces roseus</i>	15		23.1	20		30.8	11		15.7	20		26.7
	<i>Streptomyces cinnaborinus</i>	15		23.1	15		23.1	22		31.4	20		26.7
	<i>Streptomyces griseus</i>	0.0		0.0	5		7.6	7		10	8		10.7
	<i>Streptomyces sp.</i>	0.0		0.0	0.0		0.0	0.0		0.0	2		2.8
80	<i>Streptomyces antibioticus</i>	26	90	28.8	30	100	30	33	110	30	25	110	31.8
	<i>Streptomyces aureofciens</i>	20		22.2	25		25	26		23.6	30		24.4
	<i>Streptomyces roseus</i>	14		15.5	15		15	17		15.5	0.0		0.0
	<i>Streptomyces cinnaborinus</i>	14		15.5	13		13	15		13.6	18		16.4
	<i>Streptomyces griseus</i>	12		13.3	7		7	10		9.1	18		16.4
	<i>Streptomyces sp.</i>	4		4.4	10		10	9		8.2	9		8.1

Table 6: Colonization of thermophilic actinomycetes of different compost heaps at different composting periods. Data are expressed as the mean number of colony / agar plate of five replicates.

Comp. periods (days)	Bacterial sp.	Comp. A			Comp. B			Comp. C			Comp. D		
		No.of colony	Total	Freq. %	No.of colony	Total	Freq. %	No.of colony	Total	Freq. %	No.of colony	Total	Freq. %
0	Thermo dichotomicus	7	8	87.5	8	10	80	9	12	75	10	12	83.4
	Thermactinomyces sp.	1		12.5	2		20	3		25	2		16.6
3	Thermo dichotomicus	10	10	100	9	12	75	10	13	76.9	12	15	80
	Thermactinomyces sp.	0.0		0.0	3		25	3		23.1	3		20
20	Thermo dichotomicus	20	50	40	25	52	48.2	25	55	45.5	27	55	49.1
	Thermo vulgaris	20		40	18		34.6	25		45.4	25		45.5
	Thermo sp.	10		20	7		13.2	5		9.1	3		5.5
40	Thermo dichotomicus	20	40	50	27	42	64.3	22	40	55	22	42	52
	Thermo vulgaris	20		50	10		23.8	18		45	20		48
	Thermo sp.	0.0		0.0	5		11.9	0.0		0.0	0.0		
80	Thermo dichotomicus	10	20	50	10	20	50	13	23	56.5	12	23	52.2
	Thermo vulgaris	10		50	10		50	10		43.5	11		47.8