ORIGINAL PAPER 원저

미생물 및 생화학적 질량역적분석에 의한 퇴비화단계별 부숙도 평가

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Assessment of Compost Maturity on Their Different Stages with Microbial and Biochemical Mass Dynamics

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ABSTRACT

Microbial and related biochemical mass of composts are important for optimization of its process and end-products. This study was carried out to assess the specific microbial and related biochemical mass which could be used as an indicator for compost maturity during composting stages. The samples from five compost plants were collected at three stages (Initial, Thermophilic and Mature) and analyzed for total aerobic bacteria (TAB), Coliforms, *Escherichia coli*, *Actinomycetes* and fungi. Significantly, the coliforms and *E.coli* counts decreased during the thermophilic stage and were completely eliminated during the mature stage. However, the other microbial mass were not eliminated during mature stage. Which disclosed that Coliforms and *E.coli* communities can be used as compost maturity indicator. Interestingly, the microbial biomass carbon and nitrogen ratio (MBC/MBN) were decreased a little during the thermophilic stage due to the decreasing number of coliforms, *Ecoli* and fungi, while the ratio increased during the mature stage due to increasing fungal and aerobic bacterial counts. In addition the heavy metals were shown strong negative correlation with *Actenomycetes*. This study provides insight to the evaluation of compost maturity as well as the quality by the metal-microbial interactions.

Keyword : compost, thermophilic, coliforms, microbial biomass nitrogen, maturity

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초 록

유기물의 퇴비화과정중 미생물과 이에 관련된 퇴비의 생화학적 질량의 변화는 퇴비화공정 최적화와 최종산물의 품질은 매우 중요하다. 본 연구에서는 퇴비화단계중 미생물과 관련 생화학적 변수의 질량변화가 퇴비부숙도의 기준으로서의 적합성을 평가하였다. 전국 5개 퇴비공장 (용인축협, 양평축협, 논산축협, 전주연초, 지리산낙협)에서 세 단계 (초기, 부숙, 후숙)의 퇴비시료를 채취하여, Total Aerobic Bacteria(TAB), Coliforms, ***Escherichia coli***, ***Actinomycetes***, Fungi 등의 군집농도를 분석하였다. 연구결과, 5개 퇴비공장의 시료에서 Coliforms과 ***E.coli*** 는 부숙단계에서 급격히 감소되어 후숙단계에서는 완전 사멸되었으나 다른 미생물은 완전 사멸되지 않았다. 그러므로 Coliforms과 ***E.coli*** 군집을 부숙도의 기준으로 제시하였다. 미생물탄소질량/질소질량비 (MBC/MBN)는 부숙단계에서 약간 감소하였으며, 후숙 단계에서는 증가하였다. 이는 부숙단계에서 Coliforms, ***E. coli***, Fungi 등의 군집감소 때문으로, 후숙단계에서는 Fungi 및 TAB 군집증가 때문으로 이해된다. 또한 중금속성분 농도는 방선균 군집과 매우 강한 음(陰)의 상관성을 나타내었다. 본 연구의 성과는 Coliforms과 ***E.coli*** 군집 농도를 퇴비부숙도 기준으로 제시하였으며, 중금속농도와 미생물군집농도 상관관계를 이용하여 퇴비품질의 평가기준을 제시한 데 있다.

1. Introduction

Compost is a product of aerobic process during which microorganisms play an important role. Essentially, microorganisms decompose the organic matter into a stable amendment for improving soil fertility[1),2)]. During composting, microorganisms use the organic matter as a food source and produces heat, CO_2, water vapor, humus as a result of the growth and activities of different population of microbes like bacteria, fungi, Actinomycetes[3)]. An adequate maturation is essential for an effective and safe utilization of compost in agriculture, since soil amendment with poorly stabilized compost could adversely affect both crops and the environment[4)]. The development of analytical methods for assessment of compost maturity, both reliable and easy to determine, is important to better control the compost quality. The success of the composting process, however, relies on the quantity of the aerobic microbial community. The operating strategies, optimal moisture and turning frequency varies significantly depending on the type of manure used, would influence the composting process and time of maturation[3),5),6)].

The assessment of compost has focused on compost maturity to determine the composting strategies to optimize the process and produce high quality end-product[2)]. Most of the criteria used in the evaluation of the composting process and compost maturity were based on physical and chemical parameters of organic material, it reflects the metabolic activity of different microorganisms involved in composting process which depends on the quantity of microbial populations. The microbial activities and mass are key parameters that can also be used to elucidate the compost maturity[7)]. The changes in physico-chemical properties during the composting process have been studied extensively[8)]. Nonetheless, the degree of maturity and quality of the compost can be determined by several factors such as organic matter evolution, the size of compost microbial biomass, and heavy metals. This microbial biomass is a sensitive biological parameter measured in compost, which varies with the perturbation of the environment. Many studies have shown that microbial biomass size varies with type of the culture system, fertilization, organic amendments[9] and of heavy metals[10]. Most of these studies have been restricted to monitor the changes in microbial activities during composting. A few attempts have been made to determine the correlation between chemical mass and the microbial communities with compost stages[11),12)].

This paper includes five microbial populations quantity (total aerobic bacteria, coliforms, *Escherichia coli*, fungal and *Actinomycetes*) and basic biochemical parameters (including microbial biomass carbon, nitrogen and heavy metals) from five compost plants. The dynamics of these parameters and their association with compost stages were evaluated to identify the most suitable indicator of compost maturity relating microbial community and chemical parameters.

2. Materials and Methods

2.1 Compost samples

The samples were collected from five compost plants in South Korea [Table 1] during three characteristic composting stages, including initial (pretreatment or mixing) thermophilic (composting) and mature (end-product). During sampling, each pile temperatures were checked at the five different points within depth of 60 cm. Samples with the mass of approximately 500 g, were collected and mixed well in a sterile plastic bag, and stored at 4℃.

2.2 Biochemical analysis

Compost samples were analyzed for moisture content at 105℃ for 24 h, total Kjeldahl nitrogen (TKN), organic matter (OM) by loss on ignition at 550℃ for 5 h. The OM concentration of the compost was computed from the ash matter by using equation 1 and 2.

$$\text{Ash Matter (g/kg)} = \frac{\text{Ash weight of compost (g)}}{\text{Dry weight of compost (kg)}} \quad \cdots\cdots (1)$$

$$\text{Organic Matter (g/kg)} = 1000 - \text{Ash weight of compost (g/kg)} \quad \cdots\cdots (2)$$

MBC and MBN were also analyzed by the chloroform fumigation-extraction method[13),14)]. Duplicate compost samples (20 g) were fumigated with ethanol-free $CHCl_3$ for 24 h and samples were extracted with 0.5M K_2SO_4. Unfumigated composts samples were also sampled at the time the fumigation commenced. Organic carbon in the compost samples was measured using the Potassium Dichromate Oxidation Method. The MBC was

[Table 1] Characteristics of compost plants

Symbol	Location	Raw materials	Method of composting
YP	Yang-pyung	Pig waste + sawdust	Mechanical stirring
YI	Young-in	Pig waste + sawdust	Mechanical stirring
CC	Chun-ju	Plant waste + pig waste + sawdust	Aerated static pile
JM	Jiri Mountain	Dairy waste + sawdust	Aerated static pile
NS	Non-san	Pig waste + sawdust	Mechanical stirring

estimated using the following equation[13),14)].

$$MBC = FBC - UFBC/0.35 \quad \cdots\cdots\cdots\cdots (3)$$

Where FBC : Fumigated biomass carbon (g C kg^{-1} dry wt)

UFBC: Unfumigated biomass carbon (g C kg^{-1} dry wt)

The MBN from the same compost extract samples were determined as TKN using the Kjeldahl digestion procedure and calculated using equation 4[13),14)].

$$MBC = FBN - UFBN/0.68 \quad \cdots\cdots\cdots\cdots (4)$$

Where FBN : Fumigated biomass nitrogen (g TKN kg^{-1} dry wt)

UFBN: Unfumigated biomass nitrogen (g TKN kg^{-1} dry wt)

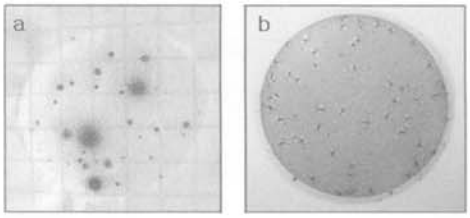
Heavy metals (Cr, Ni, Cu, Zn, Pb) were extracted by nitric acid digestion and analyzed using ICP-AES.15)

2.3 Microbiological analysis

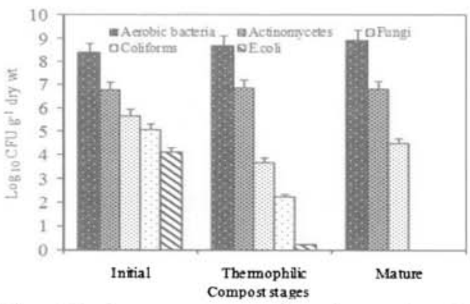
20 g of compost samples were transferred to 180 mL of sterile distilled water containing sodium pyrophosphate (0.18%) and were mixed well for 10 min and then left to settle the solids for 5 min. 10 mL of this solution added into 90 ml of sterile 25% Ringer solution (NaCl 2.25 g^{-1}, KCl 0.105 g^{-1}, $CaCl_2$ 0.045 g^{-1}, $NaHCO_3$ 0.05 g^{-1}, Citric acid 0.034 g^{-1}) and Subsequently serially diluted up to 10^{-9}. 1 mL of each dilution added to the Aerobic Count Plates and *E.coli*/Coliform Count Plates (3M Petrifilm™) in duplicate. Petrifilm were incubated at 35℃ for 48 h to aerobic count [**Fig. 1a**], 35℃ for 48 h to coliforms and 35℃ for 24 h to *E.coli* [**Fig. 1b**] according to manufacture instruction. The fungal counts, 0.1 mL of each dilution was spread on Rose Bengal Agar (Difco) plates (2 plates for each dilution) fortified with chloramphenicol at 100 g mL^{-1}. These plates were incubated at 25℃ for 5 days. The *Actinomycetes* counts, 0.1 mL of each dilution was spread on Starch-Casein-Nitrate agar (SCN) fortified with Rose Bengal (0.035g L^{-1}) and cycloheximide (50 g mL^{-1}). These plates were incubated at 27℃ for 12 days.

2.4 Statistical analysis

Box plots were constructed to provide a visual summary of the distribution of microbial data collected from five compost plants at three stages of composting as shown in [**Fig. 6**]. The graphs were plotted using SPSS[16)]. Pearson product moment correlation coefficients were calculated to show relationship between microbial mass and biochemical mass [**Table 3**] to determine the most important parameters for compost maturity.



[Fig. 1] Aerobic microbes. (a) coliforms and *E. coli* colonies (b) of composts on 3M Petrifilm™



[Fig. 6] Average mass of various microbial communities of composts at different stages of composting.

3. Results and Discussion

Solid composting is a self-heating, aerobic, solid-stage, biodegradative process of organic waste. The composting process at the microbial level involves several interrelated factors, namely metabolic heat generation, temperature, aeration, moisture content, and available nutrients. The microbial and chemical properties of compost are int errelated with type of composting and maturation process even in different composting strategies used[11]. Based on temperature, the process of composting can be sequenced into three major stages, a mesophilic-heating stage (2-4 days, initial), a thermophilic stage (20-45 days, composting) and a cooling stage (56-120 days, maturing)[11]. Based on this, microbial and biochemical properties of compost and their interrelated quantities of composts were analyzed at three different stages to explore suitable compost maturity indicators.

3.1 Biochemical analysis

The amounts of different chemical parameters were summarized in **[Table 2]**. The average temperature was observed at 27.2℃, 59.9℃ and 26.2℃ on initial, thermophilic and mature stage of compost respectively. The range of temperature was ranged at 24.6 ~ 30.2℃ on initial, 54.9 ~ 65.7℃ on thermophilic and 22.2 ~ 30.1℃ on mature stages of compost. The moisture contents widely varied from 32.7 to 68.1% during initial stage. However the thermophilic and mature stage maintained at narrow range at 42.5 ~ 48.6% and 51.7 to 56.5% respectively. The average moisture content was measured at 51.4%, 44% and 56% on the three proceeding stages of composting. For efficient composting, moisture content must be maintained constantly at about 60% with a 4-day turning frequency[11] for mechanical mixed composting system. The OM was continuously decreased from the initial to the mature stage, due to microbial degradation of the OM and transformation in to nitrogen

[Table 2] Chemical Quantity Profiles of the Compost Samples on their Characteristic Stages

Chemical Profiles	YP			YI			CC			JM			NS		
	I[a]	T[b]	M[c]	I	T	M	I	T	M	I	T	M	I	T	M
Temperature (℃)	29.1	56.7	24.3	24.6	65.7	22.2	27.5	62.2	30.1	30.2	59.8	28.4	25.4	54.9	25.9
Moisture (%)	68.1	46.6	51.7	51.4	44.4	56.5	63.8	42.8	51.8	32.7	42.5	51.7	40.9	43.6	70.7
Organic Matter (g kg^{-1})	805	848	764	805	768	719	817	778	751	796	695	667	801	751	694
Total Nitrogen (g kg^{-1})	12.2	14.9	16.1	25.6	29.2	30.8	27.6	25.4	28.4	21.8	28.6	37.1	40.8	47.8	41.8
MBC (mg g^{-1})	1.44	2.3	2.73	1.25	1.63	2.06	5.45	6.62	7.86	5.62	6.59	7.83	2.96	4.28	6.23
MBN (μg g^{-1})	220	440	390	370	590	350	510	750	480	480	670	320	310	420	200
Cr (mg kg^{-1})	6.6	5.5	7.2	5.7	4.8	10.9	9.1	4.9	10.9	2.4	6.7	4.7	3.9	3.7	4.0
Ni (mg kg^{-1})	5.6	6.1	7.0	7.3	6.6	6.4	5.8	4.5	6.5	3.6	6.8	7.0	6.9	7.3	7.4
Cu (mg kg^{-1})	111	114	111	192	142	121	53.7	49.6	57.6	62.8	105	128	123	173	144
Zn (mg kg^{-1})	595	530	460	357	364	343	182	169	239	176	304	337	420	488	537
Pb (mg kg^{-1})	11	9.6	9.4	11.5	10.1	11.7	2.6	5.7	11.3	3.4	6.6	7.9	10.4	6.9	12.5

[a]I= Initial
[b]T= Thermophilic
[c]M= Mature, Compost Standard in Korea (mg kg^{-1}) = Cr <150, Ni <25, Cu <200, Zn <500 and Pb <75.

components through oxidation, nitrification and denitrification, which generate thermophilic stage during composting[17]. In case of TKN, insignificant decrease in the end products led by the depletion of organic matters.

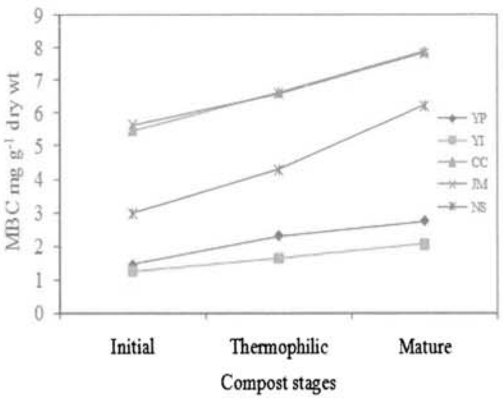
3.2 Microbial biomass carbon and nitrogen analysis

Generally the MBC was increased during the initial to the mature stage as shown in [**Fig. 2**]. The maximum and minimum MBC was observed in mature stage of CC at 7.86 mg g^{-1} and 1.25 mg g^{-1} of YI, respectively. However MBN, unlike the MBC, increased during the thermohilic stage and decreased in the mature stage [**Fig. 3**]. The highest MBN was observed at the themophilic stage and lowest at the mature stage of CC at 750 μg g^{-1} and NS 200 μg g^{-1}, respectively. Interestingly the ratio of MBC/MBN revealed a peculiar trend as shown in [**Fig. 4**]. The ratio, decreased a little during thermophilic and increased widely in the mature stage. The MBC and MBN showed the similar behavior which increased from initial to thermophilic stage, however the MBN decreased in the mature stage. This behavior as the same as those of previous work[18),11),19),20),21),22)]. This manner probably relates to the availability of readily decomposable substrates, when microorganisms are presents with a substrate and normally multiply rapidly until the substrate is nearly exhausted and the numbers reach a maximum. Thereafter, the

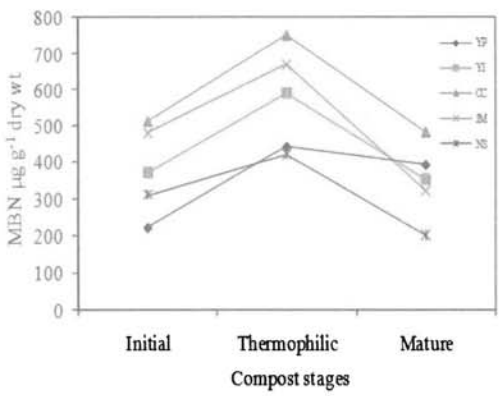
[Table 3] Pearson Product-moment Correlation Coefficient (r) and Probability (P) Values Between Different Microbial mass and Chemical mass of Composts

Microbial count	Temperature		OM		MBC		MBN		MBC:MBN		Ni		Cu		Zn		Pb	
	r	*P*-Value	r	*P*-Value	r	*P*-Value	r	*P*-Value	r	*P*-Value	r	*P*-Value	r	*P*-Value	r	*P*-Value	r	*P*-Value
Aerobic Bacteria	0.10	0.736	-0.23	0.407	0.22	0.440	0.49	0.063	-0.06	0.829	-0.43	0.106	-0.39	0.147	-0.62*	0.013	-0.36	0.182
Coliforms	-0.04	0.886	0.67**	0.006	-0.59*	0.021	-0.14	0.633	-0.53*	0.040	-0.17	0.556	0.26	0.359	0.13	0.645	-0.01	0.969
E.coli	-0.45	0.090	0.54*	0.037	-0.41	0.133	-0.32	0.241	-0.30	0.283	-0.19	0.504	0.14	0.620	-0.01	0.981	-0.02	0.935
Actinomycetes	0.11	0.686	0.18	0.517	0.33	0.226	0.49	0.067	-0.06	0.834	-0.69**	0.004	-0.67**	0.006	-0.68**	0.005	-0.55*	0.033
Fungi	-0.65**	0.009	0.15	0.590	-0.21	0.454	-0.48	0.068	0.06	0.846	-0.01	0.980	0.16	0.580	-0.06	0.831	-0.24	0.395

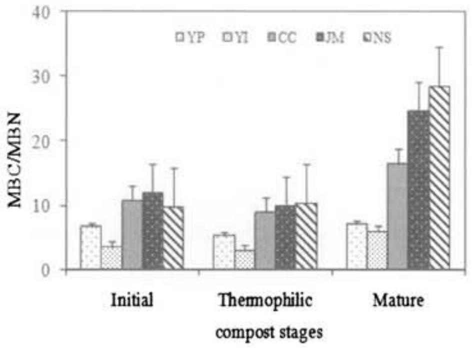
*, **, Correlation is significant at 0.05, 0.01 level, respectively



[**Fig. 2**] Dynamics of MBC with different stages of compost samples.



[**Fig. 3**] Dynamics of MBN with different stages of compost samples.



[Fig. 4] Dynamics of the MBC/MBN ratio with different stages of compost sample.

exhaustion of these substances caused by the intense microbial activity and by leaving humification, where the microbial biomass stabilizes. A similar trend of microbial biomass during composting was reported[18),19)].

The MBC/MBN ratio suggests a shift in the composition of microbial populations during compost stages. Bacteria and *Actinomycetes* populations are reported to have a protoplasmic C/N ratio of 5, whereas fungi have a ratio of 10[23)]. The results suggested a change from an initial population in which bacteria and *Actinomycetes* were prevalent, towards a final community structure, the fungi number had increased. These results were in good agreement with the general dynamics of compost microbial populations reported by previous work[19),23)].

3.3 Heavy metals analysis

As shown in **[Table 2]**, the metal contents are very different according to stages, this possibly on the source of compost, however concentrations increased during mature stage. In general, the Cr decreased in the thermophilic and increased in the mature while Ni and Zn increased throughout all stages. No trend was observed in Cu and Pb. The over all metal contents were slowly increased as the composting proceeded. However, the contents are under the limit of compost standards Korea[24)] for agricultural use except NS (Zn 537.1 mg kg^{-1}).

This would be expected from the composting mechanisms, where the organic matter is lost during the composting process due to the release of CO_2 and other volatile molecules so that the metals became more concentrated[10)]. Information of metal contents in composts of their characteristic stages can provide an insight into the metal-microbial interaction and would help in the evaluation of the quality of compost. This would facilitate the exploitation of composts in remediation of heavy metal contaminated land[10)].

3.4 Microbiological analysis: Aerobic bacteria, Coliform and *E.coli*

As a biological process, composting involves many microorganisms[25)], of which composition and magnitude, are important components of the composting process. Bacteria are usually involved in self-heating during stage of composting and utilize simple, easily degradable organic substances in the compost[25)].

The mass of different microbial communities were observed on different stages of compost and summarized as shown in **[Fig. 5]**, **[Fig. 6]**. The populations of aerobic bacteria fluctuated between 7.49 to 9.83 log_{10} CFU g^{-1} in all stages as shown in **[Fig. 5a]**. Significantly as shown in **[Fig. 5b]**, the coliform population was not observed in all samples at the mature stage. The maximum count was observed at 6.56 log_{10} CFU g^{-1} during the initial stage of YI. Coliform count was dramatically decreased from initial to mature stage and depleted at final stage. Noticeably, ***E.coli*** was not observed through the thermophilic to the mature stage. The maximum count of 5.4 $\log_{10}$ CFU g^{-1} was observed in YI at initial stage.

The ability to tell whether compost is mature or not, that is important to compost producers, plant operators and end users. Unstable and immature compost can sustain high microbial activities. Thus, when such compost is used as a soil amendment, it may deplete oxygen concentration in the soil and immobilize nitrogen, thereby causing serious N-deficiencies in crops[26]. Strictly speaking, maturity of composts is often associated with microbial activity[22),27]. The number of total aerobic heterotrophs of manure compost was high in all stages, marginally more in the beginning of composting, due to the fact that the manure contained large amounts of partially decomposed feces and urine, which contain high bacterial population[6]. Despite of differences in the components of the compost samples and composting strategies, the coliforms and ***E.coli*** count were decreased from the initial to the mature stage, thus this parameters can be used to evaluate a compost maturity. These indigenous bacteria decomposes the organic matter and transform the N components through oxidation, nitrification and denitrification[17),22] which generate heat during composting, that kills pathogenic microbes like E.coli and coliforms during thermophilic stage[28]. After the period of elevation, the temperature gradually decreased to ambient levels. At this stage, the decomposition of organic matter in the compost is more stabilized. Hence less heat is released and the microbial quantity is also stabilized but the diversity may be changed during the mature stage than others.

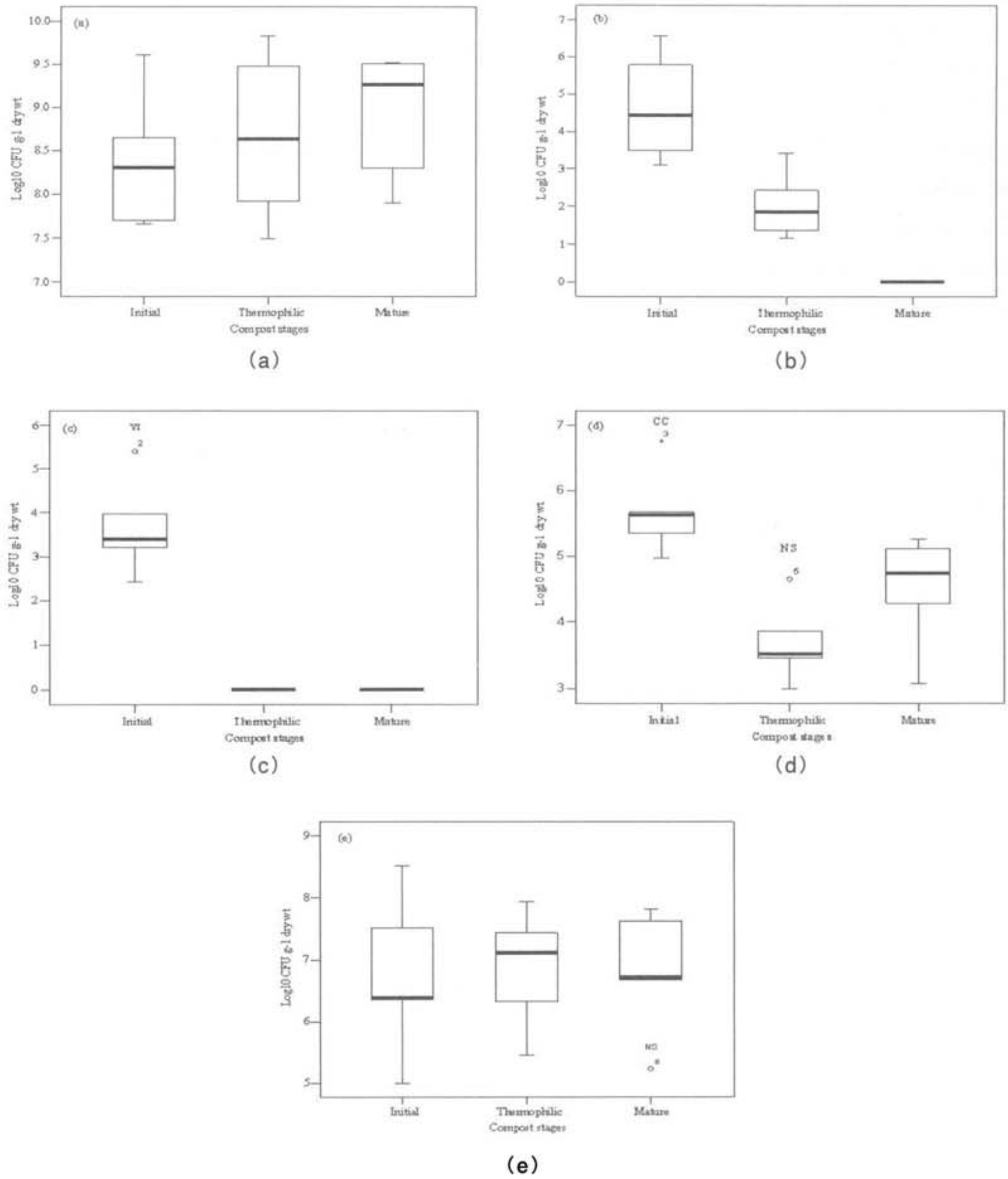
3.5 Microbiological analysis : ***Actinomycetes*** and Fungi

Actinomycetes populations were fluctuated between 5.23 to 8.53 $\log_{10}$ CFU g^{-1} the same as aerobic count with the stages. Fascinatingly these counts increased marginally in the thermophilic stage. While the fungal populations were higher in initial and mature stage, while it reduced during thermophilic stage. The maximum fungal population of 6.76 $\log_{10}$ CFU $g^{-1}$and minimum of 3 $\log_{10}$ CFU g^{-1} were observed in the initial and the thermophilic stage of CC, respectively.

Fungi also play a part in the initial rise in compost temperature[29] and most fungi were eliminated by high temperatures[3] but commonly recovered when temperatures are moderate. Hence fungi reveled the negative correlation with temperature in our study [**Table 3**]. The maturity of compost is related to the microbial activities during composting, which degrade the volatile organic matter and nitrogenous compounds in the compost. This process resulted in increase of aerobic and *Actinomycetes* count during thermophilic stage as shown in [**Fig. 5a,e**]. Towards the end of composting, no further decomposition is taking place as counts became stabilized. Composting is a very complicated process involving intensive microbial activity and the detailed mechanisms of the process have yet to be fully understood.

3.6 Statistical Analysis

Box plots were constructed to provide a visual summary of the distribution of microbial data collected from five compost plants at three characteristic stages of



[Fig. 5] Total number of various microbial communities on characteristics stages of composts and box plots showing the distribution of microbes at different stages of composting.

(a) Aerobic bacterial count, (b) Coliforms count
(c) *E.coli* count, (d) Fungal count
(e) *Actinomycetes* count.

composting as shown in [**Fig. 6**]. Pearson correlation was calculated to show relationship between microbial and biochemical mass as shown in [**Table 3**] to determine the most important parameters for compost maturity. It revealed coliforms were positively correlated with OM at r = 0.67, P = 0.006, whereas it negatively correlated with MBC and MBC/MBN at r = −0.59, P = 0.02 and r = −0.53, P = 0.04, respectively. ***E.coli*** also showed positive correlation with OM at r = 0.54, P = 0.037. Interestingly *Actinomycetes* revealed strong negative correlation with heavy metals of Ni, Cu, Zn and Pb at r = −0.69, −0.67, −0.68, −0.55 and P = 0.004, 0.006, 0.005 and 0.033, respectively. While aerobic bacteria negatively correlated with Zn at −0.62 r, 0.013 P. The fungi proved negative correlation with temperature at r = −0.65, P = 0.009. It clearly indicated *Actinomycetes* and aerobic bacteria were interacted with metals and transformed.

4. Conclusions

Based on this study it was concluded, the microbial abundance and related chemical composition changed significantly during composting stages. Despite differences in the components of the compost material and composting strategies, the coliforms and ***E.coli*** count were decreased drastically from the initial to the thermophilic and eliminated in the mature stage, thus these parameters can be apply to evaluate as a indicators for compost maturity. Total aerobic bacterial count, fungal and *Actinomycets* counts were not supportive to indicate compost maturity but the diversity of their species on characteristic stages could support. The results of MBC and MBN perception to composting substrates are also valuable because such parameters provide useful information regarding the characteristic stages on composting while the ratio can also be considered as a compost maturity indicator. To better understand the maturity of compost more physiochemical and microbiological data need to be obtained during characteristic stages.

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