

## **CHEMICAL AND MICROBIOLOGICAL CHANGES DURING COMPOSTING OF PLANT RESIDUES AND ASSESSING THE MATURITY AND STABILITY OF THE PRODUCT**

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**ABSTRACT:** *Three piles of compost were subjected to investigate the chemical and microbiological changes of some chopped plant residues (rice straw and maize stalks) supplemented with different organic amendments (farmyard manure), mineral additives (urea, bentonit, and rock phosphate) and bio-fertilizers (Trichoderma harizianum, Trichoderma viridi, and Bacillus polymyxa, Serratia sp) to prepare enriched compost. After 90- day composting course, the end product has an acceptable degree of the main physio-chemical properties such as bulk density, pH, EC, C/N ratio, and content of phosphorus, cellulose, hemi-celluloses percentages and total soluble carbon, as well as product has high microbiological activity which exhibited through studing the dehydrogenase activity, CO<sub>2</sub>- evolution and total count of bacteria, fungi, and actinomycetes. In addition, the examined maturity indices exerted that this product has a reasonable degree of maturity for germination index, NH<sub>4</sub><sup>+</sup>/NO<sub>3</sub><sup>-</sup> ratio as an indicator of nitrification process, as well as decrease of fulvic acid, water soluble carbon (WSC) and extinction co-efficient (E<sub>4</sub>/E<sub>6</sub>) and increase of humic acid all contribute to compost maturity.*

**Key Words:** *Compost preparation, chemical, microbiological, change, stability, maturity.*

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### **INTRODUCTION**

Egyptian soils are very poor in organic matter content which dose not exceed 2% and oftenly less than 1% due to arid climate and dominancy of basic soil conditions, leading to a high decomposition rate of applied manures, particularly in desert soils. These soils suffer from shortage in organic matter content as well as insufficient organic sources. Moreover, the sustainable supply of organic matter to soils is very essential concern, particularly under the intensive cropping system of Egyptian agriculture.

Disposal wastes are a problem that a great importance in modern society for its economic and environmental implications. Recycling of organic residues in agriculture is by far more efficient than the other strategies of waste management like landfill or incineration (Seque, 1996). Rice straw is produced in huge quantities in Egypt resulting in tremendous environmental

problems such as the black clouds due to its burning in open fields (Abdel-Wahab, 2008).

Composting is one of the technologies of integrated waste management strategies, used for recycling of organic materials into a useful product. It can be defined as the biological decomposition of waste organic materials to a humus-like, stable product under controlled, aerobic conditions, to be used for soil improvement and disinfection from pathogens and weed seeds (Rynk, 1992). Application of compost to agricultural land is a practice, which gains importance, particularly due to its beneficial properties on improving soil fertility, plant growth and reducing soil erosion and desertification (Gigliotti *et al.*, 2005).

One of the important factors affecting the successful use of compost for agricultural practices is its degree of maturity and stability. If unstable or immature compost is applied, it can induce inhibit seed germination, reduce plant growth and damage crops by competing for oxygen or causing phytotoxicity to plants due to insufficient biodegradation of organic materials (Brewer and Sullivan, 2003). Because of these concerns, extensive researches have been conducted to study the composting process and to evaluate methods used to describe the stability and maturity of the produced compost prior to its agriculture use (Wu *et al.*, 2000 and Pullicino *et al.*, 2007).

Compost stability refers to the rate or degree of organic matter decomposition expressed as a function of microbial activity and evaluated by means of respirometric measurements (Iannotti *et al.*, 1994) and /or by studying the transformation in the chemical characteristics of compost organic matter (Sanchez-Montero *et al.*, 1999).

Organic matter evaluation is referred to the decomposition of organic compounds and simultaneous synthesis of humic-like substances during the composting process (Gigliotti *et al.*, 2005).

Maturity is defined as the capacity of compost to support plant growth and is related to the presence of phytotoxic compounds, produced during the active composting stage and is generally evaluated through seed bioassay (Zucconi *et al.*, 1985 and Bernal *et al.*, 1998).

A number of criteria and parameters have been proposed for testing compost stability and maturity, all of which express these characteristics as a function of composting time independently of composting process or feed stock composition. The concentration of dissolved organic carbon which consistently decreases during the composting process and therefore relates to the process of stabilization (Bernal *et al.*, 1998 and Pullicino *et al.* 2007).

The aim of this investigation was to study the physio-chemical and microbiological changes during different stages of decomposition course of various plant residues, and use these parameters to evaluate the compost maturity and stability.

## MATERIALS AND METHODS

### Preparation of compost piles:

Three compost piles were prepared at Ismaillia Agriculture Research Station, Ismaillia Governorate during the season of 2005/2006. Rice straw and maize stalks were chopped to a size less than 5mm and supplemented with some amendments, as follows:

1. 150 kg rice straw + 30kg farmyard manure + 15kg bentonite + 7.5kg rock phosphate +7.5 kg urea +1.5 kg elemental sulfur.
2. 112.5 kg rice straw + 37.5kg maize stalks + the abovementioned amendments at the same rates.
3. 75kg rice straw + 75kg maize stalks+ the abovementioned amendments at the same rates.

To accelerate the decomposition rate of composting process, the composted recipes were inoculated with mixture of *Trichoderma harizianum* and *Trichoderma viridi* cultured on commercial liquid media at a rate of one liter/ton of composting materials. Fungal inoculation was made at beginning and after 30 days later. At maturity stage some bio-activating bacteria namely, *Bacillus polymyxa* and *Serratia* sp. were incorporated in to composting materials as a liquid form, at a rate of one liter of the mixture per one ton compost. The main characteristics of the raw materials used are shown in Table (1).

Heap turning was made every 15 days and moisture content was kept within 40-60 % along the composting course. Temperatures were fluctuated from 50-60<sup>0</sup>C during the bio-oxidative phase (within the core and its adjacent sites), then decreased to 40-50<sup>0</sup>C during curing stage.

Representative samples had been taken after 30, 45, 60, 75, and 90 days from three places selected along heap top, middle and bottom, the collected samples were air dried and then prepared for determining physio-chemical and microbiological characteristics of composts, as well as its maturity and stability indices.

### Procedures of analyses:

- Physical properties (temperature, bulk density) were determined according to Iglesias-Jemens and Perez-Garcia (1989) and Culley (1993).
- Seed germination was assayed using cress seeds (*Lepidium sativum* L., local variety) to evaluate compost maturity (Zucconi *et al.*, 1981).
- E<sub>4</sub>/E<sub>6</sub> ratio (extinction coefficient) was measured colourimetrically at 465 and 665 nm wave length in aqueous extract (Page *et al.*, 1982).
- Chemical characteristics were determined as described by Page *et al.* (1982).

**Table (1): Some characteristics of used raw materials**

Property	Rice straw	Maize stalk	Farmyard manure	Rock phosphate	Bentonite
pH (in susp.)	5.83	7.20	7.88	7.78	7.90
EC (dsm <sup>-2</sup> )	4.90	6.11	3.61	3.15	5.16
TOC (%)	51.78	52.49	13.22	0.39	0.15
TN (%)	0.51	0.53	0.91	0.031	0.029
TP (%)	0.016	0.122	0.56	10.86	0.38
C/N ratio	101.52	99.03	14.52	12.58	5.17

TOC=Total organic carbon

TN=Total nitrogen

TP=Total phosphorus

- CO<sub>2</sub> evolution was assayed according to Wu and Ma (2001).
- Cellulose and hemi-cellulose were determined according to Low (1993).
- Humification index (HI) was determined using the method of Bernal *et al.*, (1998).
- NH<sub>4</sub><sup>+</sup>-N and NO<sub>3</sub><sup>-</sup>-N were determined by steam distillation procedure using Mg-Devarda alloy method according to Black *et al.* (1965).
- Microbiological examinations were determined as described by Page *et al.* (1982).

## **RESULTS AND DISCUSSION**

### **A- Some physical and chemical changes during the composting process:**

Changes in some physical and chemical properties that occurred during the composting process are presented in Table (2). Data revealed that bulk density gradually increased in all of the studied piles by progressing the composting process. Values of the bulk density increased from 0.13 to 0.35 g/cm<sup>3</sup>; 0.11 to 0.37 g/cm<sup>3</sup> and from 0.14 to 0.35 g/cm<sup>3</sup> for piles I, II and III respectively. Bulk density of the compost would be expected to increase due to break down of the materials, resulting in more compact compost. After composting completed, the particle size became smaller, as a result of breaking cellulose and ligno-cellulose compounds. However, the increasing of compost weight per unit of volume. This is a good indicator for compost maturity (Raviv *et al.*, 1987). Increasing the bulk density indicates the reduction of raw materials volume as a result of decomposition process (Rynk *et al.*, 1992 and Abdel-Wahab, 2008).

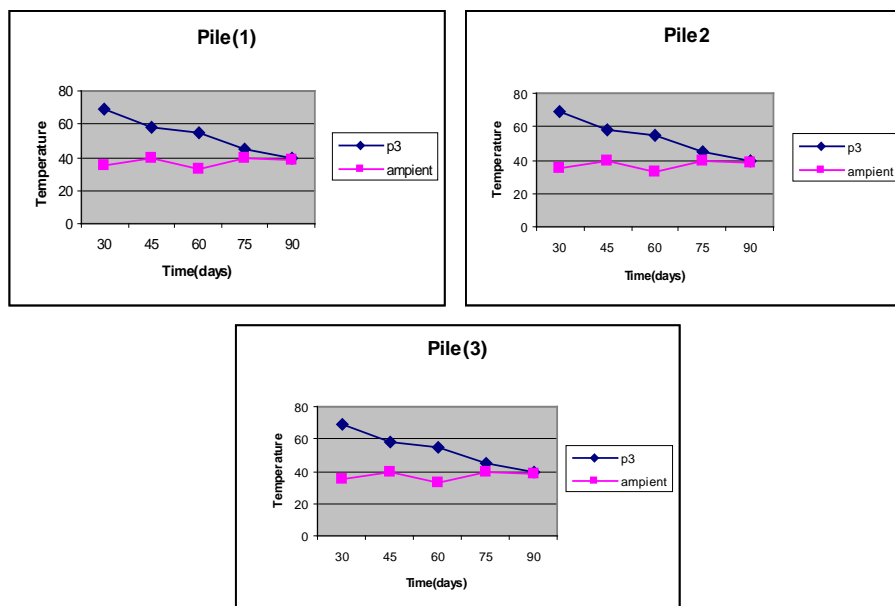
Temperature has been widely used as one of the most important parameters for evaluating compost stability. Changes in temperature at various stages of composting are shown in Fig. (1). Results exerted that the temperature degree reached maximum (65 to 67<sup>0</sup>C) after 30 days, which represented the active bio-oxidative stage of the composting process and its remaining above 40c for 75 days in the three studied piles. Temperature status reflects the microbial activity and thermophilic bio-oxidative.

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Temperatures of the piles diminished by progressing the decomposition time to become below 40 °C after 75 days of the process in the all piles. This finding considered as an indication of alleviating the active stage of composting. Establishments of these thermophilic conditions are resulted from microbial respiration and are indication of adequate feed stock composition for aerobic microbial degradation.

**Table (2): Changes occurred in physical and chemical characteristics during the composting process**

Compost Pile	Time (days)	Bulk density (g/cm <sup>3</sup> )	pH (1:10)	EC (1:5)	TN (%)	TOC (%)	C/N	TSN	TP (%)	Cellulose (%)	Hemi-cellulose (%)
1	30	0.13	6.19	5.74	0.67	45.22	67.46	184.9	0.62	37.01	22.09
	45	0.15	7.59	5.56	0.81	37.48	63.27	184.0	0.61	31.08	16.38
	60	0.20	7.42	4.32	0.99	29.66	29.95	155.3	0.65	26.34	11.36
	75	0.32	7.47	6.04	1.06	24.84	23.43	188.6	0.69	17.29	7.92
	90	0.35	7.50	6.10	1.16	22.13	19.07	159.1	0.72	16.21	6.12
2	30	0.11	6.20	5.60	0.75	38.68	51.57	178.7	0.63	40.91	23.17
	45	0.16	7.64	5.79	0.93	33.66	36.19	173.5	0.56	31.56	17.45
	60	0.21	7.38	5.14	1.06	29.13	25.48	159.6	0.67	24.66	12.10
	75	0.34	7.52	6.76	1.22	23.88	19.57	198.5	0.62	16.62	8.44
	90	0.37	7.60	6.80	1.31	21.15	16.14	161.0	0.71	15.33	7.67
3	30	0.14	6.19	4.83	0.89	36.91	41.47	179.1	0.64	40.49	24.92
	45	0.19	7.80	4.62	0.98	31.93	32.58	172.9	0.64	31.09	18.08
	60	0.25	7.60	5.49	1.01	28.10	27.82	160.4	0.62	21.47	12.33
	75	0.35	7.87	6.83	1.25	22.69	18.15	200.7	0.62	15.12	8.71
	90	0.38	7.85	6.90	1.30	20.90	16.07	176.0	0.73	14.11	7.15
L.S.D	Heap	N.S	0.037	0.204	N.S	2.206	1.277		N.S	0.453	N.S
	Period	0.023	0.044	0.237	0.142	2.547	1.477		0.044	0.521	1.364
	Interaction	N.S	0.075	0.409	N.S	N.S	N.S			N.S	2.364



**Fig. (1): Changes in temperature at the various stages of composting piles.**

Pullicino *et al.* (2007) reported that compost pile temperature is related to microbial activity and to the rate of decomposition during composting. Hence, the obtained temperature profile reflected the relevant conditions for self-heating to produce mature and stable compost. The present findings are in accordance with those obtained by Raut *et al.* (2008) and Abdel-Wahab *et al.* (2009).

Concerning the chemical characteristics of composts, data presented in Table (2) show that pH values were below 7 at 30 days of composting, then increased to remain above 7 by elapsing the time of composting course in all studied piles. Ammonification is responsible for rising pH as a consequence of ammonia evolution (Wang *et al.*, 2004 and Raut *et al.*, 2008).

In respect of electrical conductivity (EC), its values fairly increased throughout the composting process in the three tested piles, which could be related to the release of soluble salts due to the mineralization of composted materials (Abdel-Wahab & Ahmed, 2003 and Hellal, 2007).

The total organic carbon (TOC) exhibited gradual decreases in its contents with the progress of composting process. Initial (TOC) of the three piles varied from 45.22% to 36.91%, then reached 20.90% to 22.13% at the end point. Carbon dioxide liberation is resulted from degradation of organic materials during the composting process (Goyal *et al.*, 2005). In this concern, Atkinson *et al.*, (1996) reported that about 29% of carbon was lost as carbon dioxide during composting of poultry litter with sawdust.

Data of total nitrogen (TN) (Table2) showed that its contents at the beginning of process varied from 0.89% to 0.67% for the three piles. During composting process these values gradually increased, due mainly to the reduction in weight of compost materials, as well as to the biological N<sub>2</sub>-fixation by the introduced nitrogen fixers at curing stage (Abdel-Wahab, 1999).

In relation to total soluble-N (TSN), data exerted that their values increased at the first stages of composting process, and then decreased at the active stage (thermophilic stage). However, the total soluble-N exhibited more decreases again at the end of process. The increases occurred in soluble-N at the first stages are indicative of mineralization, while volatilization as NH<sub>3</sub> was responsible for their decreases at the active stages. In addition, decreasing of soluble nitrogen at maturation stage might be resulted from the leaching of NO<sub>3</sub><sup>-</sup> and volatilization NH<sub>4</sub><sup>+</sup> (Gomez- Brandon *et al.*, 2008).

Values of C/N ratio exhibited significant gradual decreases during the composting process for the three studied piles. More over, presence of maize stalk with rice straw resulted in lower C/N ratio than pile contained rice straw only. Similar trends were obtained by Bentio *et al.* (2003) and Abdel-Wahab *et al.* (2009).

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Values of total phosphorus showed a gradual increasing during the processing course for all studied heaps; this increase was due mainly to the loss of carbon dioxide emission (Vuorinen and Saharin, 1997).

Lingo-cellulose, the most abundant compound in agriculture wastes is recalcitrant macromolecule, and consequently, its degradation is essential for the operation of the global carbon cycle (Tuomela *et al.*, 2000).

Changes occurred in the main components of the cellulose fraction such as cellulose and hemicelluloses, during composting are shown in Table (2). Results revealed that cellulose and hemicelluloses exhibited gradual significant decreases as the composting process progressed in the three tested piles. On the other hand, there was no clear effect on the degradation of ligno-cellulose components due to the mixed portions of rice straw and maize stalks. These results are in accordance with Singh and Sharma (2002). In addition, Vargas-Garcia *et al.* (2007) found that the most evident changes in cellulose fraction were observed for cellulose and some specific modification for hemi-celluloses while, the concentration of lignin remained of almost constant content throughout the process in most cases.

### **b-Some maturity and stability indices:**

Some maturity and stability indices of compost piles are presented in Table (3). The germination index (GI), which combines the measure of relative seed germination and relative root elongations of cress seeds is the most sensitive parameter used to evaluate the phytotoxicity and degree of maturity of compost (Zucconi *et al.*, 1981). GI of the three tested piles significantly increased by progress the composting process. The GI values increased from 20.3, 23.2 and 29.6 at the early stage to reach 80.0, 92.8 and 95.0 % at the end of composting time for the piles I, II and III, respectively. GI values attained for the three tested piles exceeded 50%, the threshold limit stated by Zucconi *et al.*, (1981) as an indication of phytotoxin-free compost and consequently a safe soil application. In this concern, Wong *et al.*,(2001) noted that, the low percentages of GI at the early composting stages may be attributed to the release of high concentration of ammonia and low molecular weight organic acids, which might be remediated as the composting process proceeded.

Optical density ( $E_4/E_6$ ) values of humic substances in aqueous solution showed decreases by compost progress.  $E_4/E_6$  values are referred to the molecular size condensation and aromatization of humic acid (Huang *et al.*, 2008).

**Table (3): Some maturity and stability indices of the studied compost piles**

Compost Pile	Time (days)	GI (%)	E <sub>4</sub> /E <sub>6</sub>	HA (%)	FA (%)	HA/FA	HI	WSC	NH <sub>4</sub> <sup>+</sup>	NO <sub>3</sub> <sup>-</sup>	NH <sub>4</sub> <sup>+</sup> /NO <sub>3</sub> <sup>-</sup>	DH-ase/WSC
1	30	20.3	7.98	0.34	1.85	0.18	0.75	2.850	168.94	16.03	10.53	41.14
	45	28.4	6.52	0.45	1.54	0.29	1.20	2.043	164.98	19.03	8.66	45.62
	60	55.2	4.80	1.24	0.80	1.55	4.18	1.987	125.03	30.27	4.13	35.60
	75	76.8	3.95	2.35	0.50	4.70	9.46	1.082	118.93	69.67	1.70	33.66
	90	80.0	3.20	2.92	0.43	6.79	13.19	1.990	100.12	58.99	1.69	17.98
2	30	23.2	7.43	0.31	2.48	0.13	0.80	3.063	162.91	15.79	10.31	38.27
	45	39.8	6.07	0.44	2.46	0.18	1.31	2.8980	152.93	20.35	7.51	31.82
	60	69.7	4.60	0.75	1.73	0.43	2.57	2.796	125.24	34.36	3.64	24.52
	75	86.9	3.71	1.81	1.25	1.44	7.57	1.893	119.10	72.40	1.64	15.59
	90	92.8	2.98	2.48	0.98	2.53	11.72	2.480	99.89	61.13	1.63	10.96
3	30	29.6	7.53	0.52	2.73	0.19	1.40	3.840	160.08	19.10	8.38	30.14
	45	48.6	5.86	0.71	1.76	0.40	2.22	3.430	152.38	20.49	7.43	25.39
	60	65.5	4.39	1.76	1.66	1.06	6.26	2.463	123.63	36.84	3.35	28.84
	75	81.5	3.58	2.32	1.52	1.52	9.52	1.794	123.66	77.04	1.60	18.17
	90	95.0	2.86	2.85	0.99	2.87	13.63	2.250	107.00	69.00	1.55	13.77
L.S.D	Heap	N.S	N.S	0.208	0.110		N.S		2.66	2.043		
	Period	2.05	5.02	0.241	0.127		5.001		3.078	2.357		
	Interaction	N.S	N.S	0.417	0.219		N.S		N.S	N.S		

GI=Germination index      E<sub>4</sub>/E<sub>6</sub>=Extinction co efficient      HA=Humic acid      FA=Fulvic acid  
 HI=Humification index      WSC=Water soluble carbon

Data of humic indices are shown in Table (3). Most of the piles showed increases in the humic to fulvic acid ratios (HA/FA) and humification index (HI), and decreases in water soluble carbon (WSC). Nevertheless, some differences could be observed depending on raw material. Thus, higher changes were detected in pile I between the bio-oxidative phase and all of the composting process, while piles II and III showed increases at the end of composting course. This curing stage was also the most important phase for the evolution of HI, since more significant variations on these indices could be observed at this time. These results are consistent with those previously reported in relation to transformation of organic matter to humic like substances (Wu *et al.*, 2000 and Vargas-Garcia *et al.*, 2007).

In the active initial phase of composting, the most easily available organic materials (simple carbohydrates and organic acids) is mineralized and humic type substances do not changed significantly, however, later in the maturation stage the formation of these compounds becomes unable as a consequence of microbial degradation of cellulose, hemi-celluloses and even lignin (Lynch., 1992).

In relation to the effect of inoculation on humification during the composting process, Vargas-Garcia *et al.* (2007) reported that in all cases and regardless of raw materials and bacterial inoculation, the presence of external microorganisms improved humification indices significantly.

The ratio between dehydrogenase (DH-ase) activity and water soluble carbon content (WSC) exhibited a gradually decreased during the progress of the composting time in the three tested compost piles. The values of DH-ase activity to WSC decreased from 0.41 to 0.018, 0.038 to 0.012 and from 0.030 to 0.014 for piles I, II and III, respectively. These ratios are defined as "potential metabolic index" and show the relation between microbial activity



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and easily bio- degradable organic carbon (Masciandaro *et al.*, 1998). In this concern, Benito *et al.* (2003) demonstrated that, at the beginning of the process, this ratio was high due to the high microbial activity and high content of easily biodegradable organic carbon, then as the process advanced this source of energy for the microorganisms disappeared and the microbial activity declined indicating stabilization of organic matter.

The soluble forms of nitrogen (ammonium and nitrate) revealed that,  $\text{NH}_4^+$  concentration of the three compost piles increased only during the thermophilic phase, then decreased, while  $\text{NO}_3^-$  concentration gradually increased with composting progress. The initial increase in ammonium was due to the mineralization of organic compounds, whereas its decrease occurred via  $\text{NH}_3$  volatilization and conversion  $\text{NH}_4^+$  to  $\text{NO}_3^-$  through nitrification process (Bentio *et al.*, 2003 and Abdel-Wahab *et al.*, 2009). The results after maturation phase are almost similar to these obtained by Bernal *et al.*, (1998) who suggested that the maximum level to consider the end product sufficiently mature is 0.16 for  $\text{NH}_4^+$  to  $\text{NO}_3^-$  ratio.

C- Monitoring the features of some microbial activities during the different stages of composting process.

Results of Table (4) indicate that the rate of  $\text{CO}_2$  evolution significantly decreased with composting progress. Data reflected a reasonable degree of stability. These results are in accordance with Wu *et al.* (2000); and Benito *et al.* (2003) who reported that  $\text{CO}_2$  evolution rate remained nearly stable during the curing phase of composting. The decrease in  $\text{CO}_2$  evolution along with composting progress was a result of a reduction in metabolic activity, due to the decrease of readily available organic carbon (Pullicino *et al.*, 2007).

With respect to the dehydrogenase activity, data show that, the greatest reduction in its values occurred with advancing the composting process, as a result of lowering the microbial activity. In this concern, Bentio *et al.* (2003) suggested that dehydrogenase activity can be used as a microbial index to describe the composting process.

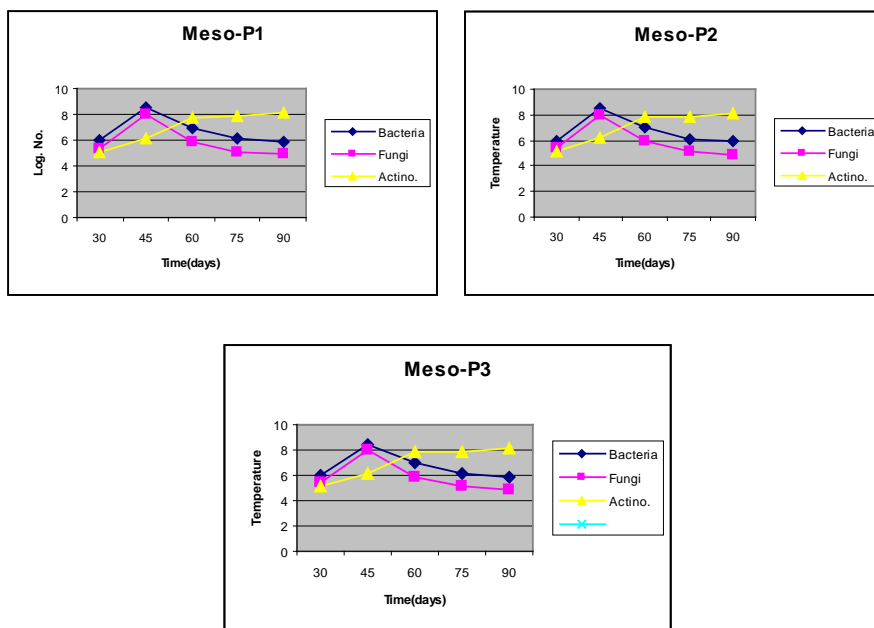
Data obtained for the total microbial counts are shown in Figs. (2) and (3). A decrease in the number of mesophilic bacteria and fungi could be observed after 45 days of composting process, while mesophilic actinomycetes were highest at 75 days of composting and increased through the curing stage.

Populations of thermophilic bacteria, fungi and actinomycetes were maximized at 30 days of composting then declined. Inoculation of compost piles with assigned microorganisms led to variations of microbial diversity along the composting process (Vargas-Garcia *et al.*, 2007).

**Table (4): Feature of the overall microbial activity during the different stages of composting**

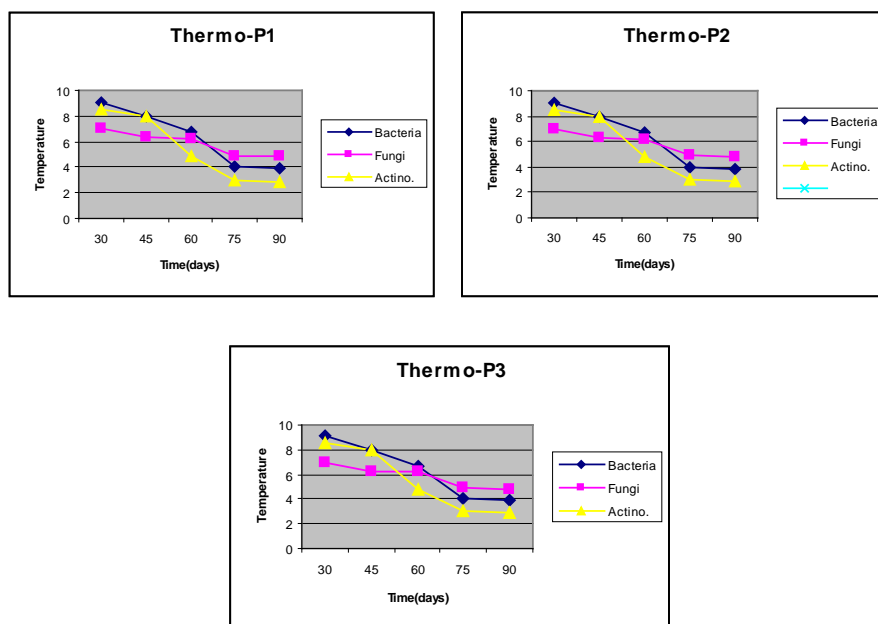
Compost Pile	Time(days)	CO <sub>2</sub> (mg/100g/24h.)	DH-ase (mgTPF/100g)
1	30	35.27	117.25
	45	20.78	93.26
	60	17.43	70.74
	75	12.54	36.43
	90	11.83	35.80
2	30	25.48	117.25
	45	19.58	91.96
	60	15.62	68.57
	75	11.83	29.52
	90	10.92	27.20
3	30	23.76	115.74
	45	16.94	87.11
	60	12.54	71.04
	75	9.90	32.61
	90	9.10	30.99
L.S.D	Heap	N.S	N.S
	Period	5.017	5.020
	Interaction	N.S	N.S

DH-ase= Dehydrogenase activity



**Fig. (2): Changes of mesophilic micro-organisms during the composting stages of compost piles.**

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**Fig. (3): Changes of thermophilic micro-organisms during the composting stages of compost piles.**

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## التغيرات الكيميائية و الميكروبيولوجية أثناء تصنيع الكمبوست من البقايا

### النباتية لتقييم نضج و ثبات المنتج

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### الملخص العربى

تم عمل ثلاثة كومبات من الكمبوست لدراسة التغيرات الكيميائية و الميكروبيولوجية لبعض المخلفات النباتية المطحونة (قش الارزو حطب الذره) و ذلك من خلال تلقيح هذه المخلفات مع بعض المحسنات العضوية و المعدنية و استخدام بعض الفطريات المحلله للمواد السليلوزية خلال مرحلة التحلل النشطة و ذلك للاسراع من معدل التحلل و اخصاب الكمبوست الناتج. و لقد اظهرت النتائج ان الخواص الفيزيوكيميائية و الميكروبيولوجية قد تطورت مع تقدم فترات التحلل حتى ٩٠ يوم . و قد اعطت عملية الخلط بين المخلفات النباتية الى الاسراع من التحلل حيث أظهرت قيم اقل فيما يتعلق بالكربون العضوى و نسبة الكربون الى النيتروجين. و بالاضافه الى ذلك فقد أعطت بعض مظاهر النضج قيما مقبوله بالنسبه لدليل الانبات لبذور الجرجير وانطلاق ثانى أكسيد الكربون و نسبة الامونيومى إلى النتراى كمؤشر لقياس عملية النترة وكذلك انخفاض نشاط انزيم الديهيدروجينيز و زيادة نسبة حامض الهيوميك و نقص كل من الفالفيك و الكربون العضوى الذائب فى الماء كدلائل على الوصول لمرحلة النضج.