Microbiological and Physico-chemical Analysis of Compost and its Effect on the Yield of Kale (*Brassica oleracea*) in Bahir Dar, Ethiopia

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ABSTRACT

The efficiency of the composting is dependent on factors such as, number of microorganisms, nutrient balance, temperature, pH, electrical conductivity and moisture content. However there is paucity of data on these parameters and the influence of compost on the yield of kale. The aims of this study were to investigate the microbial counts, the physico-chemical parameters of compost and to assess the influence of compost on the growth yield of kale (*Brassica oleracea*) plants. The number of bacteria decreased from $1.2x10^6$ cfu/g at the initial stage of composting to $1.3x10^4$ cfu/g at the end of the process. Likewise, the number of fungi decreased from $6.05x10^6$ cfu/g to $1.05x10^4$ cfu/g. On the other hand the number of actinomycetes increased from $5.2x10^5$ cfu/g to $7.4x10^6$ cfu/g towards the end of the composting period. The electrical conductivity and pH increased through out the composting period. Mean monthly moisture content, organic matter, carbon content, nitrogen content and carbon-nitrogen ratio decreased from the initial stage to the final stage of composting. Statistically significant difference in growth parameters of kale plant was observed between compost and inorganic fertilizer (p < 0.05). Electrical conductivity and pH are the parameters that influenced the growth of actinomycetes. The mature compost improved the plant growth parameters better than inorganic fertilizer Addition of compost gave a better growth of kale compared to that of inorganic fertilizers. Compost

Key words: actinomycetes, bacteria, compost, fungi, kale, physico-chemical

INTRODUCTION

Compost is the product of aerobic process during which microorganisms play an important role. Essentially, the microorganisms decompose the organic matter into a stable amendment for improving soil quality and fertility. A number of biological wastes can be used for composting which include municipal solid wastes, animal and human excreta (Tiquia, 2005). Composting has been widely used for stabilization of different types of wastes. Composting cannot be considered a new technology, but among the municipal solid waste management

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strategies it is gaining interest as a suitable option for chemical fertilizers because the process eliminates or reduces the toxicity of municipal solid waste. The final product can be used to improve and maintain soil quality (Epstein, 1997).

Compost is one of nature's best mulching and soil amendment materials. It can be used instead of commercial fertilizers. Best of all, compost is cheap because it can be produced at minimum cost. The use of compost improves soil structure, texture, aeration and soil water-holding capacity (Martin and Gershuny, 1993). Production of municipal solid waste, including organic waste is increasing while soils are progressively losing organic matter due to intensive cultivation and climatic conditions. This makes the recycling of organic waste as soil amendments, a useful alternative to incineration, landfill or rubbish dumps (Massiani and Domeizel, 1996). Recycling of organic wastes in agriculture after appropriate biological treatment can produce valuable organic matter and be of great interest in countries where nutrient-deficient and poor quality soils prevail (Hassen *et al.*, 2001). Reports showed that application of municipal solid waste compost in agricultural production has improved soil physicochemical properties, increased water retention as well as improved the crop yields (Ghaly and Alkoaik, 2010).

Bahir Dar is one of the fastest growing towns in Ethiopia facing a growing challenge of solid waste management. The per capita municipal waste generation in 2010 was 0.25/kg/day. With anticipated increase in population, it is estimated that the per capita waste generation in the year 2020 will be 0.45 kg/day (Bahir Dar Town Administration, 2010). Domestic solid waste must be effectively collected, disposed or used efficiently through composting and the compost can be used as bio-fertilizer. Monitoring of the microbial succession is important in the effective management of the composting process as microorganisms play important roles in the process and the appearance of some microorganisms reflects the quality of maturity of compost (Ryckeboer et al., 2003). Many factors such as, moisture, composition of the feed, pH, and temperature, affect the composting process and the quality of the end product (Sunberg 2005). However there is paucity of data on these parameters and the influence of compost on the yield of kale. The aims of this study were to investigate the microbiological and physicochemical parameters of compost collected from Bahir Dar town and to evaluate the influence of compost on plant growth parameters of kale.

MATERIALS AND METHODS

Description of the Study Area

The study was conducted from November, 2010 to May, 2011 in Bahir Dar town, Amhara National Region State, situated on the shore of Lake Tana at about 1780 meters above sea level. The mean annual rainfall and average temperature are 1437 ml and 18.3°C, respectively. In 1994 the town had an estimated total population of 96,140 and according to the 2010 population and housing census of Ethiopia, Bahir Dar town has a total population of 256,999 (Central Statistical authority, 2010).

Compost preparation and sampling

A composting fence having a width of one meter, length of two meters and a height of 1.5 meters above the earth was prepared. The fence was filled with solid wastes (dry leaves, stem of maize) up to 30 cm and with paper, cattle manures and food wastes above the solid waste about 20 cm height. Soil was added above the waste material for 20 cm high. Again the dry leaves, stem of maize, paper, cattle manure and food wastes (leaves of avocado, onion, orange, banana and mango) were added to the composting fence above the fertile soil. The upper layer of compost was covered with dry leaves to protect the composted heap from wind and sunshine. The other two fences were prepared with width of one meter, length of two meters and a height of 1.5 meters above the earth that is used for turning and mixing of compost (Hassen et al., 2001).

The waste was processed in open windrows of about 2 m length, 1 m of width and a height of 1.5 m. The windrows were turned once per month with a compost turner. Samples were taken from three locations (top, middle and bottom) each separated by 0.5 m vertically. A total of 30 compost samples were collected with sterilized beaker over three months period at intervals of three days for microbiological and physicochemical analyses.

Microbiological analysis

Preparation of inoculums

Fifty gram of compost was added to 950 ml of normal saline and homogenized for 30 minute. Ten fold serial dilutions $(10^{-1} \text{ to } 10^{-4})$ were made from the homogenate. The homogenate was used for enumeration of aerobic mesophilic bacteria, fungi, and actinomycetes.

Enumeration of aerobic mesophilic bacteria

One ml of homogenate was aseptically transferred onto plate count agar (Oxoid, England) in triplicates. The pates were incubated at 37°C for 24 h under aerobic atmosphere (Rebollido *et al.*, 2008). After incubation, the number of colonies were counted with a colony counter and recorded as cfu/g of compost.

Enumeration of fungi

One ml of homogenate was aseptically transferred onto saboured dextrose agar (Oxoid, England) in triplicates and plates were incubated at 25°C for 3 days. After incubation isolated colonies of fungi were counted by colony counter and recorded as cfu/ g of compost (Rebollido *et al.*, 2008).

Enumeration of actinomycetes

Half ml from each dilution was transferred and spread on half-strengthen tryptic soy agar (Oxoid, England) and incubated at 55°C for 120 h. After incubation isolated colonies of actinomycetes were counted with a colony counter (Rebollido *et al.*, 2008).

Physico-chemical analysis of compost

The temperature of the compost piles was monitored everythree days during the process using thermometer. The average reading was recorded. The ambient temperatures were also measured and recorded at the same time according to Ryckeboer *et al.* (2003). Electrical conductivity and pH of compost was measured with a multimeter (Trantmann, 1992). Samples of each composting heap were taken to the laboratory for moisture content determination. Each sample is weighed using digital balance. The samples were then oven-dried at a temperature of 110°C for 24 hours and reweighed. The amount of moisture was calculated from the difference in the weight of the compost before and after drying (Trautmann, 1992). Nitrogen content was determined by the Kjeldahl method. The carbon-nitrogen ratio was calculated using the results obtained from carbon and nitrogen contents. The carbon content of compost was determined by titration method as described in Sahilemedhin and Bekele (2000).

Plant-growth parameters of kale

Kale a green leafy vegetable is largely produced and consumed by Bahir Dar town population and therefore chosen for this study. Nine beds each with the dimension of 4 m² were prepared for the cultivation of the kale. Compost was uniformly spread on each of the first three beds, 10 g of fertilizer (NPK) was uniformly spread on each of the other three beds and the other three were used as controls. The average fresh biomass of a kale was determined by randomly selecting five plants from each bed, weighing them on a digital balance. The average dry biomass was also determined by drying five samples of kale from each plot in an oven at 105°C for 24 h. Plant height, leaf diameter, leaf length measured were measured and leaf number was counted following the procedures indicate in Baffour-Asare (2009) and Tregurth et al. (2010).

Data analysis

Data was analyzed with SPSS version 16. The microbial counts of compost were transformed to log values. Spearman's correlation was used to check if there is correlation between microbiological and physicochemical parameters. Significant differences between mean values of growth parameters of kale were determined following one-way ANOVA using Duncan's Multiple Range test (p < 0.05).

RESULTS AND DISCUSSION

The aerobic mesophilic bacterial counts in the initial phase of composting was 1.2 X 10^6 cfu/g and decreased to 1.3 X 10^4 cfu/g in the last phase of composting. The number of fungal count was high at the beginning of composting process (6.05 X 10^6 cfu/g) and decreased at the end of composting period to 1.05 X 10^4 cfu/g. On the other hand, the number of actinomycetes recorded was 5.2 x 10^6 cfu/g and 7.4 x 10^6 cfu/g in the initial phase and the last phase of compost, respectively (Figure 1). The decrease in microbial count may be due to the depletion of nutrients in the waste, accumulation of toxic products and unfavorable growth environment (Kowalchuk *et al.*, 1999).

During initial phase of the composting process, the temperature of the compost was near to ambient temperature and the pH was slightly acidic (Figure 2). The temperature of all compost regularly dropped off gradually towards during the maturation as reported by Rashad *et al.* (2010). The gradual fall in temperature and bacterial and fungal counts towards the end of process indicate the depletion of nutrients and the process approaching stability, hence less heat was generated in the compost (Kutsanedzie *et al.*, 2012)

Mesophilic fungi and bacteria are the dominant active degraders of fresh organic waste materials. Food waste containing vegetable residues often have a low initial pH (5-5.6), which stimulates the proliferation of fungi (Ryckeboer *et al.*, 2003). Actinomycetes are commonly identified as one the main groups of actinomycetes responsible for organic matter conversion during latter stages of composting. Actinomycetes compete with others organisms for nutrients and can inhibit microbial growth due to the production of antibiotics, lytic enzymes or even by parasitism (Rebollido *et al.*, 2008).



Figure 1. The aerobic mesophilic bacteria, fungal and actinomycete counts of compost over three months.

Actinomycetes play an important role in the degradation of natural polymers process and colonize organic materials after bacteria and fungi have consumed easily degrade fractions in the maturation phase. Their enzymes enable them to degrade tough debris such as: woody stems, bark or newspaper (Chopra 2004). The interaction between various functional roles of microorganisms depends on the availability and abundance of nutrient resources and their acquisition (Insam *et al.*, 2002).

Moisture content during composting process decreased gradually throughout composting process from 58.6% to 47.2%. The changes of temperature in the compost and ambient temperature over the 90 days indicated that the temperature reached to its highest temperature 56°C after six days. After four weeks it started to decline and become stable until the 90th day (Figure 2). The decrease in moisture content throughout the composting period was related to changes in temperature. This could be as a result of moisture loss through evaporation at high temperature (Finstein et al., 1986). In composting process, heat is built up in the heap which is enough to vaporize moisture from the heaps and as temperature increases, more heat is lost. This could be due to water being utilized by the living organisms present in the compost. A study carried out by Richard et al. (2002) indicated that, water provides a medium for the transportation of dissolved nutrients required for metabolic and physiological activities of organisms in the compost.

The extent of organic matter decomposition at any particular time is related to the temperature at which composting takes place and the chemical composition of the organic substrate undergoing composting (Levi-Minzi et al., 1990). The compost heaps reached their highest temperatures within the first two weeks of composting and maintained temperatures above 45 °C for almost four weeks this result is similar to Baffour-Asare (2009). High temperature is attained due to the presence of readily degradable carbon compounds, most of which initially decompose rapidly. Thereafter, decomposition rate slows down because of the greater resistance of the remaining carbon compounds (lignin and cellulose) to decomposers. Generally, the higher the lignin and polyphenolic content of organic materials, the lower their decomposition rate (Palm and Sanchez, 1991). The differences in the temperatures recorded imply the succession of different microbial communities in all the systems as reported in Miller (1993) and hence different rate of decomposition as indicated by Sundberg (2005).



Figure 2. The patterns of change in moisture content and temperature

Electrical conductivity of the compost increased from 0.57 ds/m in the initial stage to 1.5 ds/m at the final stage of composting process and the variation of pH among different aged compost samples showed that during the early stage of decomposition the pH was below 5.0 and then it continued to increase to 7.6 in the 3rd month (Figure 3). The pH begins to rise due to the release of ammonia marking the beginning of proteolytic process (De Nobili and Petrussi, 1988). The pH value of compost is considered as an indicator of process of decomposition and stabilization. The change of pH value during composting is quite predictable (Miller, 1993). The pH value settles to 7.5~8.0 as the compost stabilizes. But pH value is not an absolute reliable indicator of maturity and stability (Chefez et al., 1998).



Figure 3. Changes in pH and electrical conductivity during composting process.

The organic carbon decreased from initial month to the end of the process. Like organic matter content the youngest compost (one month old) showed the maximum organic carbon content (35.6%) and the oldest compost (three month old) was quite low in organic carbon (16.6%). Baffour-Asare (2009) reported a similar pattern of reduction in organic carbon. The total N content of one month old compost was 1.87% which continued to decrease to 1.7% during the second month and it also declined to 1.55% during the third month old compost as a result of microbial activity as reported by Baffour-Asare (2009). The carbon to nitrogen ratio decreased from initial month to the end of the process (Figure 4). It was reduced from 19.7 to 10.7. This indicates that increase in temperature is of crucial importance for efficient mineralization, which in turn results in reduced carbon-nitrogen ratio.



Figure 4. The variation of organic matter, carbon content and total nitrogen contents of compost.

Organic matter is decomposed and transformed to stable humic compounds (Amir *et al.*, 2004). The losses of organic matter is usually substantial during the biooxidative stage, when the temperature and the microbial activity are high, simply because that the organic matter provides the major energy to microbial metabolisms. This could be because most of the easy biodegradable substances have been depleted by microorganism during the first stage of the composting stage, when composting process is stable and mature; the activities of microorganisms decrease as the result of lack of energy. The reduction of nitrogen content could be due to the utilization of inorganic nitrogen by bacteria in the composting process and the conversion of nitrogen into bacterial proteins. Again, nitrogen loss could be attributed to organic nitrogen being mineralized by microbial activity during decomposition. The mineralization rate is reduced in the process, as a result of the rapid conversion of the more labile organic nitrogen, leaving the most resistant organic nitrogen in the organic nitrogen pool which takes a lot of time to mineralized (Iglesias-Jimenez and Alvarez, 1993). In addition, nitrogen could be lost through volatilization of gaseous ammonia during mixing and processing of the compost heaps. For example, nitrogen losses ranging from 9 to 68% have been reported during the composting of cattle manure (Eghball et al., 1997).

The results of correlation analysis among microbiological and physicochemical and level of risk parameters are shown in table 1. There was statistically significant positive correlation between bacterial and fungal counts and organic matter content, C:N ratio (R > 0.815, p < 0.01), actinomycetes count and pH and electrical conductivity (R > 0.9.03,

		Microbial groups		
Physical parameters	Bacteria	Fungi	Actinomycets	
Compost temperature	0.200	0.505	-0.654	
Moisture content	0.595	0.682	-0.468	
Organic Matter	0.815	0.888	-0.884	
Organic Carbon	0.854	0.908	-0.892	
Total Nitrogen	0.623	0.790	-0.871	
Carbon to nitrogen ratio	0.806	0.866	-0.858	
pН	-0.795	-0.923	0.903	
Electrical Conductivity	-0.798	-0.929	0.939	

Table 1. Correlation coefficients (R2) between microbial counts and different physical parameters of compost

	Parameters plant ⁻¹				
Treatments	Plant height (cm)	Leaf length (cm)	Leaf diameter	Leaf number	
			(cm)		
Compost	36.91±2,83a	23.15±2.65a	18.05±1.23a	8.8±1.2a	
Chemical fertilizer	29.09±1.61a	16.78±1.45b	13.16±1.07b	6.4±0.7b	
Control	18.87±1.63b	11.82±c	7.48±0.76c	4.3±0.9c	
F values	46.2	140.4	249.9	52.4	

Table 2. Plant growth parameters of kale plants treated with compost and chemical fertilizer

Each value is the mean of 10 replicates. Mean values followed by different letters are significantly different within a row or column at $p \le 0.05$ according to Duncan Multiple Range test (DMRT)

Table 3. Plant growth parameters of kale plants treated with compost and chemical fertilizer

Treatments	Fresh biomass* (g)	Dry biomass* (g)
Compost	120.44±0.87a	10.52±0.97a
Chemical fertilizer	99.17±1.10b	8.04±0.68b
Control	58.15±1.33c	5.45±0.33c
F values	8020.1	127.9

Each value is the mean of 10 replicates. Mean values followed by different letters are significantly different within a row or column at $p \le 0.01$ according to Duncan Multiple Range test (DMRT). *Biomass of the whole plant.

p < 0.01). Electrical conductivity and pH are the parameter that influenced growth of bacteria and fungi and the organic matter content, C:N ration were the parameters that influenced the growth of actinomycetes. The results of this study are in agreement with the finding of Robellio *et al.* (2008).

Growth parameters of kale plants

The matured compost had a positive effect on plant height, leaf length, leaf number, and leaf diameter of Kale (Table 2). Similar results were obtained by Bernal *et al.* (1998) where the addition of organic materials improved soil texture and quality and increased water holding capacity and infiltration; increased workability and reduced erosion (Carter and Stewart, 1996). In this study the extension of composting period has an important role on mineralization of compost mixed with soil as a longer period could supply more nitrogen resulting in a positive effect on the growth and development of plants. Tregurth *et al.* (2010) showed that higher concentration of soil organic matter in the compost treatments contributed to the improved waterholding capacity of the soil and improving the soil's ability to hold water has important implications for high-yielding crops like kale.

This study showed that plots receiving compost held up to 9% more water than the control. There was statistically significant difference in the growth parameters of kale plants grown with compost compared to those with grown in chemically fertilized soil (p=0.01) (Table 2). Priyadarshani et al. (2013) showed that treatment with compost had had significant effect on the shoot and root dry weights, number of leaves and number of tillers.

CONCLUSION

The aerobic mesophilic bacteria and fungi decreased while actinomycetes count increased at the last stage of composting. Electrical conductivity and pH are the parameters that influenced growth of bacteria and fungi negatively while the organic matter content, C: N ration were the parameters that influenced the growth of actinomycetes. Addition of compost gave a better growth of kale compared to that of inorganic fertilizers. This study was conducted on only one type of composting process that is municipal solid waste composting, so further work on different composting types would reveal new insights to find out the best compost to improve both soil quality and plant growth.

ACKNOWLEDGEMENTS

The authors would like to thank Bahir Dar University for supporting the study.

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