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Microbial Ecology of Composting Dead Poultry and Their Wastes

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Abstract: Composting is environmentally acceptable disposal route, with potential financial benefits. Emerged Avian influenza virus epidemics in Egypt 2005-2009 potentiate proposing composting as sound method.Constructing a newly designed movable closed composting unit for dead poultry with AIV H5N1 and their wastes was one of main project goals for hygienic disposal. Field litter samples before composting confirmed existence of 4 species of Gram negative and 3 species of Gram positive bacteria as well as Aspergillus species with absence of anaerobes. Efficient composting was attained at temperature ranged 40-60°C, relative humidity 60-74%. The litter carbon content ranged from 43.77-54.72% with mean 49.25 % and carbon: nitrogen ranged (C:N) from 21.54-24.33%. Composting reduced total colony count 80 % and total fungi count 66.10%, Salmonella Spp. and Clostridium spp. count (70.59 and73.68% respectively). End compost had the highest C: N value 24.33%, moisture content 22.34%, total nitrogen 2.21%, total phosphorus 0.54 % and total potassium 0.79%. Compost product is used for agronomic purpose (1:2) after had been subjected to chemical and microbial examination. The well-grown edible obtained vegetable had no phototoxic impact to pose health risk. Composting is recommended for hygienic disposal of dead birds and their wastes with more environmental safe level than traditional methods used in Egypt.

Key words: Composting · Litter · Bacteria · Fungi Load · Thermal Profile · Anaerobes · Environmental Safe Level

INTRODUCTION

Composting is a biological process in which organic wastes are stabilized and converted into a product to be used as a soil conditioner and organic fertilizer. During composting, mesophilic bacterial growth is stimulated by the higher temperatures. The elevated temperature induces thermophilic bacterial growth. The pattern is then repeated in a second hotter stage. The process is self-limiting because of excessive accumulation of heat which will eventually fall. Anaerobic digestion of poultry manure has been shown to be a viable disposal option [1]. Operating conditions are important, as excessive levels of ammonia and/or high pH or temperature levels can inhibit methane production [1]. These microbial activities require a carbon:nitrogen (C:N) ratio between 15 and 25, a moisture content of 40 to 60%, a pH between 5 and 12 and greater than 30% free air space. For poultry waste, a low C/N ratio contributes to large ammonia losses.

Composting provides an inexpensive alternative for disposal of all dead animals, including poultry. Environmentally acceptable, disposal routes, with potential financial benefits are required. The temperatures achieved during properly managed composting will kill or greatly reduce most pathogens, reducing the chance to spread disease. Properly composted material is environmentally safe and a valuable soil amendment for growing certain crops [1]. Composting has proven to be an effective, environmentally sound method of dead bird disposal. Rodents, scavenging animals and other pests are seldom a problem. Fly larvae, pathogenic

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bacteria and viruses are destroyed during composting [2]. Most of the N present in poultry waste is organic in nature and a large part of it is derived from protein. A management tool that would allow environmentally safe disposal of poultry wastes coupled with satisfactory crop yields would be very useful [3].

Compost water content range is 45 - 65% w.b, pH 5.8-9.0 and temperature 45-60°C as reasonable ranges. Microorganisms in composting include; the mesophilic 10°C - 43°C (50°F - 110°F) and thermophilic 43°C-71°C (110-160°F) which are the principal groups [4-8].

The current work was designed to investigate the efficacy of newly designed composter for disposing poultry and their wastes infected with avian influenza H5N1 as well, obtaining compost product with more environmental safe level with the most important pathogens for agronomic use.

MATERIALS AND METHODS

Collection of Field Samples:

- Water samples were collected from main water supply for broiler or layer farms as well from drinkers inside farms (representative samples from different sites).Sterile containers were used for water viral, bacterial and fungal isolation. Water samples for viral isolation were kept in freezer at -4°C until accomplished.
- Freshly dead birds were collected from farms suspected (symptomatically) or previously diagnosed via referee lab. for being infected with AIV H5N1 by their owners. They were quickly transferred through cooled vehicle and subjected to layering within the previously prepared composting unit.
- Litter samples were collected parallel to water samples collection on the same farms for microbial investigation. Poultry waste samples consisting of broiler litter, manure from laying operations and dead bird composts were collected across Cairo, Almenofya and Alfayoum governorates. Poultry waste samples were collected during 2010-2011. Bedding materials encountered were sawdust and straw. Collection of bulk samples from poultry wastes of poultry houses was carried out. Random sub samples of poultry wastes from poultry housing or waste storage facilities were collected and combined to yield a 0.5 m3 composite bulk sample of each poultry waste [3].

Procedures

Construction of Newly Designed Movable Closed Composting Unit: The composting unit (200W x150Lx180 D cm) was designated by the principle investigator after reviewing many of the movable composter produced worldwide. The proposed design with its special requirements to fulfill the most environmental safe level when transferred to field trial was manufactured by National Research Center (maintenance devices sector in cooperation with STDF Egypt, finance sector). Because the composting was carried out in boxes, natural aeration did not occur. It is though necessary to install an artificial aeration system. Air could be blown into the compost via interior installed small fan fixed on side –wall of the composting manufactured unit. In some of the installations, compost temperature was controlled by aeration and the aeration cycle with preset aeration times that changed as a function of degree of maturity of the compost. Composting in boxes was carried out for a short period of time (6-8 weeks) and a curing stage was followed outside the boxes. During second stage of composting the number and species of mesophilic bacteria increased markedly. A thermogenic phase with temperatures exceeding 60°C was even considered as a "microbial suicide" [9].

Composting Procedures

Layering: On impervious stainless steel floor of the composter place an initial layer of 30cm of fresh litter (straw) for bacteria to start decomposing process and would help absorb carcass fluids or excess water that may be added to the composter. A thin layer of bulking material such as litter cake was added (obtained from field farms and sometimes from broiler production unit in Faculty of Vet. Med., Cairo University). Composting is a biological process in which organic wastes are stabilized and converted into a product to be used as a soil conditioner and organic fertilizer, after regular depopulation without evidence of infection or epidemics. A layer of bird carcasses was arranged in a single layer side by side, touching each other. Placed carcasses were no closer than 20cm from the walls of the composter. A small amount of water might be needed after each carcass layer. A layer of built up litter was added twice as thick (20-25cm) as the layer of carcasses underneath. After completing the initial layer, subsequent layers of carcasses were added; bulky ingredient and litter until a height not exceeding 150 cm was reached. The last (superficial) layer would be a cap of 30 cm of new straw litter, modified after [2].

Monitoring Composting Process: Monitoring compost temperatures and maintaining good management practices throughout the entire process helps ensure the elimination of insect larvae and pathogens in the final product [2]. Daily monitoring of the temperature °C and relative humidity % were carried out via controlled sensors inserted internally (descended vertically from top toward bottom 60cm for thermal prop but 30cm for humidity prop) and readings were manifested on LCD. Management of temperature °C and relative humidity percentage were accomplished via blowing of fresh air (blowers or fans fitted on internal left sidewall of composter) or adding new source of carbon (wood shaving or straw) or source of nitrogen as bacterial substrate (built up litter with its organic contents) to keep the required C:N ration. Adding water (when moisture was decreased than 40%) or bulking material (when increased more than 60%) and turn pile were done [2]. Deep in the pile temperatures were monitored manually using analogue thermometers. Temperature readings were taken towards the central part of the top, middle and bottom locations of the piles. For the surface temperature, the temperature probe was plunged into the composter roof to be reached a depth of 30 cm from the top of the piles. Samples and temperature readings were taken, twice a week until the termination of composting trial [2]. Composting contents (dead birds and their litter) from different layers were randomly collected. The collected samples were thoroughly mixed and 600-g were subjected to bacterial and fungal examination and the third sample was collected and sent aseptically to biotechnology laboratory, Faculty of Veterinary Medicine, Cairo University for detection and confirmation of presence or absence of AIV H5N1 and tracing its origin. Turning the layered contents of the composter was carried out because biocidal temperatures were not reached at the outer edges of the compost unit and turning and mixing the compost at least once was needed to ensure the destruction of pathogens and nuisance insects as done in primary composting bins by Ritz and Worley [2]. End product of composting dead birds and their belongs was removed from the composter and subjected for estimation of its microbial load (bacteria, fungi and virus). Confirmed absence of pathogens (mainly H5N1 virus) was attained from biotechnology center. Then, free end product of H5N1 was spread on very thick plastic sheet beside the experimental room where the composting unit for sun dryness to get rid of remaining moisture. The obtained dried end product after 10 days dryness was stored in clean dry store room. This product was added in agronomic rate 1: 2 with normal cultivating soil from faculty green areas for planting edible green plant (watercress and mint). The well-grown plant within two weeks was harvested for laboratory investigation chemically and microbiologically before human consumption to be ensure of its safety. The use of previously examined compost product for cultivating edible vegetable was coincided with Dunkely *et al*. [10].

Laboratory Investigations:

Evaluation and Characterization of Poultry Wastes Subjected to Composting: Random samples 600-g of the compost mixes were removed from days 1- 35.

- Moisture content was measured for each subsample (50 g) portion to determine dry weight (105°C overnight) and ash content (555°C for 24h) [11]. Chemical characterization consisted of analyses for total N and organic carbon (C). Nitrogen was calculated by the Kjeldahl method [11] and carbon was determined as described by Haug, [12] and total potassium (K) by dry ashing and extraction using dilute hydrochloric acid (HCl) [13].
- Microbiological investigation, the obtained composting product was spread over very hard plastic sheet on the ground near the lab. Where composting unit was located exposed to sun and natural dryness continued for 10 days with daily turning over. The dried non smelling composting product was collected and stored in a dry room until further bacteriological examination and trial to use it for agricultural purpose. Compost analysis was examined for E. *coli*, total coliforms and *Salmonella* spp. according to Dunkley *et al*. [10]. Isolation and, identification of anaerobes mainly *Clostridium* species with regards to *C. perfringens* was carried out according to Princewill *et al*. [14], Wilkins *et al*. [15], Koneman *et al*. [16] and Monica [17]. Isolation and identification of mycotic species was done according to Nichita *et al*. [18]. Uses of compost were preceded by analysis of the product nutrients. [2]. Well-grown vegetable microbial analysis was performed; Vegetative material was weighed and 2 X volume of buffered peptone water were added. The samples were then stomached for 2 min. and soaked at room temperature for 1.5 hr. before diluting for isolation of *E. coli* Procedures for *E. coli*, total coliforms and *Salmonella* enrichment were performed as was done with the compost samples [10].

Phototoxic Bioassay: This test was performed according to the modified protocol of McLaughlin *et al*. [19]. The test samples were incorporated with sterilized E-medium at different concentrations; 5, 50 and 500 µg/ml in methanol. Flasks with methanol were serving as a negative control and reference inhibitor that is parquet was serving as a positive control.

Treatments were replicated three times and the flasks were incubated at 30°C for 3-5 days.

RESULTS AND DISCUSSION

Table 1; Litter samples collected from different poultry farms revealed existence of different Gram positive (Staphylococcus, Streptococcus, Bacillus spp and Corynebacterium spp.) and Gram negative (Salmonella spp., Shigella spp., Proteus spp., Klebsiella spp. and *E. coli*) bacteria. No anaerobes could be isolated. Mycological examination indicated dominance of Aspergillus spp., (*Asp. fumigatus, Asp. niger* and *Asp. Penicillium*). Both of Bacillus spp. and Corynebacterium spp, were.isolated from 2/7 of poultry houses litter, while Streptococcus spp, Staphylococcus spp were isolated also from 2/7 of poultry houses litter. *E. coli* was prominent in 3/7 of poultry houses litter.

Field litter samples confirmed the existence of 4 species of Gram negative and 3 species of Gram positive bacteria as well Aspergillus species with absence of anaerobes. This existence was expected from field poultry farms according to previous results of Rothrock *et al*. [20], they reported that poultry litter inside a house represents an ideal environment for microbial growth (temperature, moisture and nutrient content are well within the range for microbial proliferation). The concentrations of *Salmonella* spp. were below detection ($<5 \times 10^3$ cells/g).

Table 2: Water samples collected from main water sources were characterized by dominance of some Gram-positive (staphylococcus spp. and bacillus spp.) and Gram negative bacteria (*E.coli*) with absence of anaerobes and mycotic spp. Water samples collected from drinkers inside poultry farms revealed dominance of many Gram-positive bacteria (staphylococcus spp., streptococcus spp., bacillus spp. and Corynebacterium spp.) with absence of gram negative, anaerobes bacteria and mycotic spp. The presence of Corynebacterium in drinkers despite its absence from main water supply indicated that this drinker water has been contaminated with litter material containing dropping. This contribution was confirmed by presence of Corynebacterium spp. in litter. Water and litter samples were collected for figuring out microbial population, mainly bacterial and mycotic.

Table 1: Microbiological examination of litter from different poultry farms before establishing composting procedures.

	Litter Bacteriological examination			
Houses	Gram Positive	Gram Negative	Anaerobes	Mycological examination
1	Bacillus spp	*E. coli*	-	Aspergillus spp.
2	Streptococcus spp.	*E. coli*, Proteus spp		Aspergillus niger
3	Corynebacterium spp.	Klebsiella spp		Aspergillus spp.
4	Streptococcus spp. Staphylococcus spp.	Salmonella spp		Aspergillus fumigates
5	Staphylococcus spp.	Shigella spp Proteus spp		Penicillium
6	Bacillus spp.	*E. coli*		Aspergillus niger
7	Corynebacterium spp.	--		--

Table 2: Microbiological examination of water samples from different poultry farms

Water source #	Gram +ve	Gram -ve	Anaerobes	Fungus.spp
1-Drinkers	-	*E. coli*		
		Proteus spp	-	-
Main	Staphylococcus	-	--	--
2- Drinkers	Corynebacterium spp.	-	--	--
Main	Bacillus spp	*E. coli*	--	--
3-Drinkers	Streptococcus, Staphylococcus	-	--	--
Main	Bacillus spp	*E. coli*	--	--
4-Drinkers	Staphylococcus	-	--	--
Main	--	-	--	--
5-Drinkers	Bacillus spp, Staphylococcus	-	--	--
Main	Staphylococcus	*E. coli*	--	--

Table 3: Microbiological analysis of poultry wastes prior and post composting

Analysis	Built-up	New litter	End compost	Soil	Mix
Total colony count X 10^5 g^{-1}	6.1	4.9	2.2	7.2	9.2
Total fungi X 10^3 g^{-1}	3.1	2.8	2.0	3.2	1.4
Salmonella spp. count X 10^2 g^{-1}	1.4	2.0	1.0	1.7	2.1
Clostridium spp. count X 10^4 g^{-1}	1.8	2.0	1.0	1.0	-
E. coli. count X $10^{2\ g^{-1}}$	6.2	3.4	1.2	5.1	4.0
Phytotoxic	Non	Non	Non	Non	Non

Table 4: Chemical analysis of poultry wastes prior and after composting

Parameters	Built-up	Straw	End compost	Soli	Mix
pH	6.50	6.76	6.82	6.71	7.10
Moisture content %	24.14	15.89	22.34	55.86	38.14
Ash content %	2.3	1.5	3.2	2.5	3.6
Carbon content %					
Range 54.3. -54.72	54.28	54.72	53.77	54.17	53.55
Total nitrogen %					
Range 2.21-2.54	2.39	2.54	2.21	2.27	2.29
Carbon: Nitrogen ratio					
Range 21.54-24.33	22.71	21.54	24.33	23.86	23.38
Total phosphorus %					
Range 0.50-0.61	0.61	0.50	0.54	0.55	0.57
Total potassium %					
Range 0.79-0.88	0.88	0.82	0.79	0.84	0.87

This virus could not be detected using RT-PCR from all examined water samples despite positive characterization of avian influenza *H5* gene from some bird's lung from the examined farms [21].

Table 3 shows that the highest total colony count was obtained from built up litter (6.1x 10^5 g^{-1}) collected from poultry farms with suspected infection with avian influenza virus.On adding news straw (4.9 x 10^5 g^{-1}) with built up litter and subjected for composting with dead bird carcass,this count was reduced to 2.2x 10^5 g^{-1}. Composting reduced total colony count from 11x 10 $^1g^{-1}$ in mix of both litters (built-up and new) to 2.2 x10^1 g^{-1} in compost product (80% reduction). Total fungi count was highest in normal cultivating soil 3.2x10 $^3g^{-1}$, in built up litter was 3.1x 10 ^{3} g^{-1} while in end compost was 2.0x10^3 g^{-1}. Fungal count was reduced from 5.9 x103g^{-1} (mix of built up 3.1 and new litters 2.8) to 2.0 x10^3 g^{-1} in end compost (66.10% reduction). Composting reduced Salmonella Spp. count from 3.4 x10^2 (mix of 1.4 in built up litter and new litter 2.0 x 10^2g^{-1}) to 1.0 x10^2 in end compost (70.59% reduction). Clostridium spp. count was reduced from 3.8 x 10^4 g^{-1} (mix of built up 1.8 and new litters 2.0) to 1.0 x 10^4 g^{-1} in end compost (73.68% reduction). Current compost product did not have any bone left, therefore no expected presence of Botulinum bacteria and the threat of botulism is decreased. *E.Coli* count was reduced from 6.2x10^2 g^{-1} (built up litter) to 1.2x10^2 g^{-1} post composting with reduction 79.03%. No phototoxic substances were detected from all kinds of collected samples. The current microbial population load seemed lower than reported by previous works [22, 23] where the microbial population of poultry litter could be as high as 10^9 to 10^{10} cells per gram of litter. However, the microbial ecology during composting is affected by many factors contributing to continuous input of excrement and the resultant effects on physiochemical parameters (pH, moisture, organic N) that may affect the microbiota differently. Some works reported that the total heterotrophic counts are highest (10.3– 10.6 $\log^{10}$ MPN/ g^{-1}) at the beginning of composting. Their numbers dropped until the end of the composting trial. The initial pH of the poultry litter ranged between 8.18 and 8.33. [20, 24]. Current result range of pH value was 6.50-6.82 in built-up litter and end compost respectively, while fungal count was lower in compost Vs built-up litter. Acidified poultry litter is an excellent environment for the proliferation of fungi. Lower pH (in the range of 5 to 6) is known to inhibit bacterial populations and select for fungal communities [20, 25]. The optimal temperature for thermophilic / thermotolerant fungi is 40-50°C. The number and species diversity of moderately thermophilic bacteria are low at 40- 50°C and increase at 50-60°C [9].

Table 5: Composting thermal profile and relative humidity %

	Composting Conditions	
Days	Temp. °C	RH%
1	29	70
3	38	74
5	40	77
7	42	72
9	45	69
11	49	72
13	57	75
15	60	65
20	45	62
25	40	60

The current results (Table 5) indicated that temperature increased gradually and persisted between 40-50°C for 4 consecutive days (7th -11th days) which was destructive for most pathogens. At temperatures of 55°C for 3 consecutive days, most pathogenic bacteria and parasites are killed and most viruses are inactivated [26].

The well grown vegetable microbial profile revealed total colony count $1.2x10^2g^{-1}$, fungal count $1.1x10^2$ g^{-1} with no detection of Salmonella spp. and Clostridium spp. and no phytotoxic impact was detected. Disappeared bacteria species in end compost attributed to the thermal impact of composting process as denoted in table 4 and previously was recorded by Berge *et al*. [26]. Windrow composting of spent broiler litter resulted in at least 6 log10 reductions in numbers of total coliforms, fecal coliforms, *E coli* and fecal *Enterococcus* spp [27].

Table 4 illustrates that; compost product pH was 6.82 which was higher than built up litter 6.50. Highest pH value 7.10 was for mix of compost product and normal cultivating soil.The result coincides with that recorded by Sesay *et al*. [24] where by the end of the composting process, the pH fell to nearly neutral values (7.0), which is an indication of stabilized organic matter.

Compost moisture content was 22.34% which was lower than built-up litter 24.14%.Compost product had lowest nutrient elements level as total nitrogen was 2.21%, total phosphorus 0.54% and total potassium 0.79%. The used mix of compost and cultivating soil in agronomic ratio 1:2 had highest potassium 0.87 %. Current compost nutrient percentages revealed its well done and can be used for agronomic purpose [2, 28], where they recorded, well composted mortality can be used as a soil conditioner and nutrient source for crops just as fresh poultry litter. Compost is typically lower in nitrogen and slightly higher in phosphorus and potassium than manure and is thought to release nitrogen at a slower rate. Litter and compost were applied to fields at rates that meet crops feed nutrients. Ash % ranged from 1.5-3.6 which looked within results of composting broiler houses litter (1.5-4.0) by Brake [29]. The carbon % ranged from 43.77-54.72 with mean 49.25 % and the carbon: nitrogen ratio ranged (C:N) from 21.54-24.33 with mean 22.49%. End compost had the highest values. The obtained carbon % was higher than that recorded by Brake [29] where it ranged from 35-41.6% in most broiler houses in composting studies.But more than that recorded by Beffa [9] where normally, a C/N ratio of less than 20 in mature compost is thought to be desirable. C/N values measured in sufficiently stabilized composts vary between 5 and 20, depending on the type of raw material..Moreover, temperature has been found to be correlated with most of the important compost properties such as C/N ratio, pH [30]. The high initial moisture content (65%) hinders aeration and could induce anaerobic condition during composting [31]. During composting total P and K are increased. The NH4 +-N concentration decreases dramatically during composting. The compost made from poultry litter contains nutrients essential for plant growth, including trace elements [32].

Obtaining well grown edible vegetable when using end compost mix with normal cultivating soil in agronomic ratio 1:2 confirmed its content of essential nutrients for plant growth, this result coincided with results achieved by Tiquia and Tam [32], Wood *et al*. [33] and Flynn *et al*. [34]. Application of the composted material to soil resulted in low NH3 losses, as NH4–N concentrations were low. The study found that the largest reduction in NH3 losses from poultry excreta was achieved if the excreta were dried prior to storage and incorporated into soil [7].

During 1-15 days temperature increased from 29-60 C°, then declined to 40C° between 20-25 days. Relative humidity ranged from 60-77 % during whole composting process. This result does not coincide with Rynk *et al*. [6], who reported moisture content should be maintained between 40 and 60% during the composting process while it was involved within levels recorded by Fernandes *et al*. [35] who reported successful composting of poultry manure mixed with peat or chopped straw that has been obtained in a passive static-pile at high initial moisture levels (73–80%).

CONCLUSION

There are many disposal options for dead livestock currently in use throughout the world. On-farm disposal methods are favored by the farming community due to the perceived environmental, practical, economical and Biosecurity benefits. Compost of current work proved to be free of Avian influenza virus and of reduced microbial load. The compost used for agronomic purpose (1:2) after had been subjected to chemical and microbial examination. The well-grown edible obtained vegetable had no phototoxic impact to pose health risk. Results recommended composting for hygienic disposal of dead birds and their wastes with more environmental safe level than traditional methods used in Egypt.

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