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Effect of *Salmonella* on Decomposition of Poultry Litter

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Abstract

Chicken litter is a significant source of nutrients for production of crops and to reduce the impact of human pathogens on environment. The physicochemical properties of poultry litter mixture compost was assessed against the maturity and stability indices such as carbon nitrogen ratio (C/N), nitrate (NO_3), ammonia (NH_3), ash, Cation Exchange Capacity (CEC), Humification Index (HI), Humification Rate (HR) and Degree of Polymerization (DP). The use of *Salmonella* significantly improves the humification process and physicochemical properties of final compost product are better in comparison to control trial. The most prominent effects was the fast rise in temperature from mesophilic to thermophilic stage was accompanied by an increase in NH4+-N that gradually decrease near the maturation phase. Moreover, the decrease in carbon and nitrogen ratio and increase in DP, CEC, HI, HR, phosphorous and potassium was also observed. A significant correlation was found between the maturity and stability parameters like C: N ratio, cation exchange capacity, humification index, degree of polymerization, humification rate and nitrate. Therefore, it is acclaimed that isolated microorganisms *Salmonella* from poultry litter promote the stable compost formation.

Keywords: Polymerization; Compost; Carbon nitrogen ratio; Enrichment; Humification.

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1. Introduction

Poultry litter is a mixture of poultry feces, bedding material, wasted feed and feathers [1]. It has imbalanced amount of plant nutrient, pathogens inappropriate carbon nitrogen ratio and odor [2]. Due to decline in soil fertility and high amount of chemical fertilizers prices proper composting of organic waste for plant nutrient becomes the need of day. Composting is the low technology or low investment process that converts organic solid waste into an organic fertilizer recognized as compost [3]. Composting, is a good

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technique as compares to burning and different drying techniques which are used in recent years. The microbiological and chemical processed recycled chicken feces litter is significantly used as organic fertilizer and is widely spread on productive land [1]. Major source of nitrogen and phosphorus is poultry litter that may also offers many trace elements for the production of crop. Poultry litter not only comprises of plant nutrients such as nitrogen potassium and phosphorus but also contains trace elements such as As, Cu, Zn, pesticide deposits, microorganisms and pharmaceuticals. It is also very advantageous in improving the biological, physical and chemical fertility, representing that the usage of such valuable resource on land application always remains the central choice [4]. However, the fresh poultry litter may contain C: N ratio, pathogens, improper amount of plant nutrients, and annoying odors [2]. Poultry litter is also a source of human pathogen such as *Camphylobacter jejuni*, *Listeria monocytogenes*, *E.coli* and *Salmonella* [5] that also contaminate the environment commonly associated with food borne outbreaks. Different types of pathogens can be found in chicken litter such as *Mycobacterium, Streptococcus, Bordetalla, Campylobacter, Staphylococcus, Clostridium, Escherichia coli, Globicetella, Corynebacterium,* and *Actinobacillus.* In chicken litter *E.coli* is present with the incidence rate as high as 100% [1]. Various diseases has been caused by these pathogens [6] such respiratory problems, chicken pox, histoplasmosis, gastrointestinal helminth infection, histoplasmosis is basically caused by the inhalation of spores of the fungus *H.capsulatum* that favors moist, caves, silos and shady conditions. Compost consist of microbial components most important are bacteria and fungi. *Actinomycetes* is most important than others and perform best in presence of oxygen some other bacterial and fungal species are represented as (i.e. *Macrobrachium rosenbergii Bacillus subtilis*, and *Bacillus, Penaeus monodon* and *Licheniformis* [7], some *actinomycetes* (as *Streptomyces fradiae*, keratinolytic fungi (*Myceliophtora, Chrysosporium, Trichophyton spp.)* [8] are proficient in using native keratine as source of nitrogen, carbon and energy. Most important parameter is pH that influences the composting process and microbial activity. Initially the pH of compost is high but gradually reduced due to the decomposition process [9]. Most of the microorganism cannot survive the high pH or in a high concentration of ammonia. *Salmonella spp*. and *Clostridium spp*. are the two examples of organism that are able to withstand for long period of time under unfavorable conditions and are also able to repopulate. When microorganisms did not attain the optimal moisture level 60 % the microbial activity start to decline composting process and temperature 40–70^{o}C is not achieved. If distribution of O_2 is not homogenous due to high moisture then accumulation of carbon dioxide may occur that produces the anaerobic conditions inside the compost. Little amounts of fodder yeast preferred the growth of microorganisms but in the final product may cause sanitary risk [10]. Some environmental conditions alter the composting process such as moisture, in case of degradation of organic matter it largely affects the physical and chemical properties of litter. Effective disposal of poultry litter requires bulking agents in order to optimize the carbon nitrogen ratio. During composting process bulking agents may reduce the emission of NH_3 [11]. Composting of organic materials not only provides micro and

macro nutrients but it also develops soil characteristics such as water holding capacity (WHC), CEC and activity of beneficial microflora [2]. Improving of soil properties enrichment of microbial habitats is necessary for growth of microbes including water contents, pH and aeration conditions [12]. Rapid stabilization and bio oxidation of organic material suddenly acidifies the compost and inhibit microbial activity that produce odor [9]. Although microbes reduces the biomass but researchers proved that single microbe is unable to convert polymers into monomers due to the presence of sulfur comprising of amino acid protein [13]. The present study aim was to isolate the *Salmonella* from poultry litter to observe the physicochemical changes in the poultry litter mixture during composting.

2. Experimental

2.1. *Isolation of bacteria from poultry litter*

For the isolation of bacteria from the poultry litter freshly collected samples of poultry litter were streaked on potato dextrose agar (PDA) plates. These plates were incubated 48 to 72 h at 38 to 42°C without aeration. Colonies of different bacterial strains were recognized on these plates. Different species of salmonella were separated from this culture of bacteria on agar plate.

2.2. *Isolation of Salmonella*

Salmonella-typical colonies on the agar plates (pale or colorless with or without black centers on DCA and a bright red color on PGDA) were cultured on the agar plates having triple sugar iron agar slants, urea agar and lysine broth and incubated at 38°C for 48 h. By sub culturing onto fresh propylene glycol deoxycholate agar (PGDA) plates the purity of suspected *Salmonella* colonies were identified that were hydrogen sulfide positive on TSI agar, urease negative and lysine positive. The purified *Salmonella* colonies were moved to agar slants by streaking and further biochemical tests were performed which included sugar, decarboxylase test and glycerol fermentation.

2.3. *Salmonella spp. cell culture*

In tryptone soya broth *Salmonella* culti-loops were suspended. Tryptone soya broth (TSB) was prepared earlier by suspending 30 g in 1 L of DW in a clean flask. By autoclaving at 121°C for 15 min, the flask having the broth in it was sterilized. The cell was grown in tryptone soya broth approximately after 48 h in fridge. The cell culture was enumerated by plating appropriate serial dilutions using sterile ringer solution on tryptone soya agar (TSA) from oxide. TSA was prepared by suspending 40 g of tryptone soya agar in 1 liter of cleaned water and bringing it to the boil until it dissolved entirely and then sterilized at 121°C for 15 min. The cells that were grown in the TSB counted by plating out on tryptone soya agar immediately after incubation for 24 h at 37°C.

2.4. *Composting procedure*

Poultry litter was collected from different poultry farms and mix with corn straw of size 0.5 mm. All apparatus used for composting process were sterilized. Firstly, flask was washed thoroughly with DDW and sterilize, after adding poultry litter and corn straw in it, flask mouth was closed by putting cotton plug in its mouth and covered with aluminum foil after that it was autoclaved on 121°C at 15 psi for 15 min in order to kill all microbes in poultry waste. Three flasks were taken for control and three for experimental compost separately. In experiment, initially 100 g of each sterilized poultry waste mixture (poultry litter: 70% and corn straw (30%) substrate was used. The sterilized raw material mixed with 10% of broth inoculum of each *Salmonella* isolates, separately. During composting the inoculated flasks were incubated at 38°C. Process of composting took place in triplicates and monitored for up to 65 days. One sub sample was taken at first day of composting and every seven days regularly thereafter up to 65 days. First sub sample was stored at 4°C to proceed the analysis. During sampling pH was determined using pH meter and moisture content were determined by simple reduction of water loss.

2.5. *Analytical method*

The chemical properties (C/N), humification rate (HR), fulvic acid (FA), degree of polymerization (DP), humic acid (HA), humification index (HI), cation exchange (CEC) and ash) of compost were verified as described by [11]. The micronutrients (Zn, Mn, Fe, Cu, and Cd) compost was analyzed by using the atomic absorption spectrophotometer. The compost chemical properties were examined by using standard methods, namely, pH (1:1, compost: water) by the [14] method and compost organic carbon by the Walkley and Black method [15]. The total N and total C in compost was carried out by catalytic tube combustion using the Vario Macro elementar CHNS analyser (S.N:11046079). The carbon and nitrogen ratio was calculated as the quotient of total carbon over total nitrogen.

2.6. *Statistical analysis*

Microsoft excel used to calculate SD (standard deviation) and SPSS 19.5 was used for person correlation between CEC, DP, HR, HI, C/N and nitrate. In order to measure the analytical error and sampling, triplicates samples analyzed three times.

3. Results and Discussion

3.1. *Characteristics of poultry litter and composting mixtures*

The physical and chemical properties (moisture: 55.7%; pH: 7.6; ash: 42.45%; organic matter: 57.55%; total nitrogen: 3.78%; total carbon: 31.4%; C/N: 8.3; total P: 1.2%) of poultry litter were presented in Table 1. The moisture content impacts on composting process and poultry litter contains 55.7% which was more than bulking agent [16]. Moreover heavy metals (Zn, Pb, Cu, Mn) were also available in different ratios. The bulk

density and FAS of corn straw results were in lined with other scientific results. According to the data (Table 1), the physical structure of the material to be composted had also improved significantly with the addition of corn straw.

Table 1. Chemical characteristics of poultry litter, experimental, control compost and corn straw.

Parameters	Poultry Litter	Corn Straw	Experimental Compost	Control Compost
Moisture (%)	55.7	9.23	29.12	38.65
pH	8.4	5.50	8.39	8.78
Ash (%)	41.5	31.51	61.32	45.32
Organic Matter (%)	58.5	68.5	38.68	54.68
Total Nitrogen (%)	3.23	0.11	5.89	3.76
Total Carbon (%)	30.2	33.15	66.5	75.23
C/N ratio	9.35	301.3	11.3	20.0
Total P (%)	1.13	0.04	2.14	1.03
Total K (%)	1.03	0.01	1.98	1.23
Total Sulpher (%)	0.20	0.25	0.15	0.11
Total Hydrogen (%)	4.2	1.2	3.23	3.4
Zn (mg/kg)	19.13	0.02	16.43	12.23
Pb (mg/kg)	0.53	0.01	0.23	0.12
Mn (mg/kg)	1.01	0.01	1.51	0.03
Cu (mg/kg)	0.10	0.01	0.40	0.10

3.2. *C/N ratio*

Carbon to nitrogen ratio in Table 2 is showing the clear increase in decomposition. Experimental results depicted that with the addition of *Salmonella* further decrease in decomposition occurs rapidly. Tenth day of decomposition shows 28.1 value of C/N in control and 27.87 in experimental with no distinct difference. Similarly, on 40 days of decomposition control value of C/N reaches 25.65 and experimental value at 18.08. At the end of the graph experimental decomposition giving some stable values of C/N by different days of decomposition as compare to control values. Percentage on 40 days of decomposition is approximately 41.86%. Over time there is decrease trend in C : N ratio, corn-straw as bulking agent plays a significant role in the increase of C/N ratio. The narrow C/N ratio through the ammonium volatilization cause the loss of nitrogen in poultry litter [17]. Addition of poultry manure to corn-straw further increases the carbon and nitrogen ratio [18]. Further proceeded composting may be the source of increase C/N ratio. Vigorous ammonia volatilization is the source of increase in carbon and nitrogen ratio [17]. At the end of composting C : N ratio reduction is high [2]. Under composting condition proper aeration and moisture provided greater mineralization of organic material. Material becomes finer and softer, C : N becomes stable as the decomposed litter temperature approach the maximum temperature. Reduction in organic acid content followed by decrease in carbon and nitrogen ratio by conversion of organic C into CO_2

[19]. Existence of unutilized complex N substrate shows high carbon and nitrogen ratio while on other hand instability of compost shows low C/N ratio [19]. Reduction in C is a significant indicator of maturity of compost. Decline in the ratio is mainly due to microbial actions because microorganisms use organic compounds as source of nutrition [16].

Table 2. Effect of *Salmonella* on C/N ratio, nitrate, ammonium and ash.

Days	C/N		Nitrate		Ammonium		Ash	
	Experimental	Control	Experimental	Control	Experimental	Control	Experimental	Control
0	29.54	29.54	0	0	0	0	3.53	4.1
5	28.04	28.32	76.98	40.1	94.8	65.98	5.89	7.0
10	27.87	28.1	81.45	54.87	123.54	89.54	7.21	12.54
15	26.56	27.78	90.21	65.98	117.09	105.76	8.28	19.76
20	23.56	27.43	120.14	98.32	145.89	111.09	9.02	26.65
25	22.89	26.67	143.06	109.76	196.76	125.87	15.67	27.96
30	22.06	26.33	169.87	127.87	207.32	136.43	28.23	37.03
35	19.46	25.19	248.56	183.09	245.80	165.78	29.65	45.76
40	18.08	25.65	295.43	197.53	213.09	170.65	32.76	56.87
45	17.86	24.79	301.38	201.76	199.86	155.43	38.54	57.09
50	17.98	23.87	307.68	212.98	165.32	189.11	33.76	51.03
60	17.76	23.08	298.09	190.87	154.98	187.98	38.09	49.81
65	17.81	23.63	302.17	201.90	171.04	193.43	35.87	53.54

3.3. *Nitrate*

(NO_3^-) in Table 2 showing different values both experimental and control at different number of days. Both values are increasing continuously and constantly, control values at fifth day of decomposing showing 40.1 mg/kg nitrate and experimental values showing 76.98 mg/kg nitrate on the same day. An increase in concentration of both control and experimental values was notified. However, they were 183.09 NO_3^- (mg/kg) control and 248.56 NO_3^- (mg/kg) experimental at 35 days of decomposing. After rise, there is decrease in values of both control and experiment occurs at the same day but at the end of the experiment again showing the rise in values. Comparison of both values showing the gradual increase in values. In order to know the percentage of NO_3^- in both experimental and control values on 5th days of decomposition it gives 26.33%. Similarly percentage on thirty fifth days of decomposition is 47.90%. NH_3 volatilization may cause decline in $NH4^+$-N that indicates the increase in NO_3^-N [17]. The temperature rise may cause the loss in $NH4^+$-N and NO_3^-N that presented for N plant. Significant fall in $NH4^+$-N and NO_3^-N may occur due to the decomposition of ammonium salt, nitrate that present in sewage sludge [20]. In the soil distinctions in microbial community structure effects the rate of de-nitrification, nitrogen fixation and nitrification, large amount of nitrates also generates other complications because nitrates are salts and dehydrate their surroundings.

Because of being very strong oxidizers nitrates literally burn up the organic matter in the soil [21].

3.4. *Ammonium*

In this graph NH4-N+ indicate two values of control and experimental but both giving different values at the same day. Initially on the 10^{th} day of decomposing both values gradually increasing but on the 15 days the experimental value is going to decrease as compare to control and giving value 117.09 mg/kg at this point, control value is 105.76 mg/kg of $NH4^{+}$-N. Difference of both is approximately 11.33 mg/kg. On other hand 10 days of decomposition gives percentage about 27.52%. After fall in the experimental value steady increase in graph may occur and gives maximum value of 245.8 $NH4^{+}$-N mg/kg on the 35^{th} day of decomposing. Decrease in the concentration of $NH4^{+}$-N was due to gaseous ammonia that cause by volatilization. Similarly control value is also giving maximum value on the same day that is165.78 mg/kg of $NH4^{+}$-N. Amount of $NH4^{+}$-N rises continuously up to 15 and 35 days of decomposition in both experimental and control decomposition (Table 2). Microbial activity is the reason of metabolism of protein that may lead to the increase the conc. of $NH4^{+}$-N. Decline in NH_4^{+}-N is the reason of decomposition of nitrate and ammonium salt [20]. High $NH4^{+}$ is the reason of achieving thermophilic temperature because of higher microbial activities [22]. When thermophilic phase is achieved, accumulation of $NH4^{+}$ may occur due to an active mineralization of organic compounds.

3.5. *Ash*

Ash is substrate inorganic portion and it may contain huge variety of inorganic minerals such as Mg, Ca, Fe, and Na along with extra trace metals. The amount of ash rises regularly both in control and experimental decomposition and reach 5.89% and 7.0% in control and experimental decomposition within the 5 days. By the end of decomposition process both experimental and control values reaches to 53.54% and 35.87% in both experimental and control within the 65 days (Table 2). Ash and organic matter are inverse of each other, high organic matter contents results in low ash contents [16]. On 45 days of decomposition maximum percentage of ash given by both control and experimental that is 38.54% and 57.09% both gives comparative 32.49 percentage on 45 days. Maximum rate of decomposition shows higher microbial activities [23]. Accumulation of high ash contents in experimental trials was due to organic matter fast decomposition by microbes [24]. Furthermore, the ash content is an additional sign of decomposition process that shows a high degree of decomposition by high degree of accumulation. Intensity of decomposition represented by the difference between control and experimental substrates results [16].

3.6. *CEC*

Table 3 shows the regular increase in the decomposition of CEC (meq/100 g) with the number of days. On the 5th day the decomposition of experimental is higher than control, giving different values as 7.09 (meq/100 g) in control and 16.65 (meq/100gm) in experimental, respectively with the difference of 9.56 CEC (meq/100 g). As decomposition increases CEC due to the microbial alteration of lingo cellulose into humus [11]. On twenty-fifth day the decomposition of CEC increases and reaches to 32.76 CEC (meq/100 g) in control and 49.32CEC (meq/100 g) in experimental showing 33.57% of decomposition on 25th day by comparing both experimental and control values. With ammonium acetate CEC is identified and measured at 7 pH [25].

Table 3. Effect of *Salmonella* on CEC, humification rate, degree of polymerization and humification index.

Days	CEC		Humification Rate		Degree of Polymerization		Humification Index	
	Experimental	Control	Experimental	Control	Experimental	Control	Experimental	Control
0	7.3	5.98	2.05	1.0	0.0	0.0	0.0	0.0
5	16.65	7.09	2.43	1.21	1.1	0.90	1.3	1.40
10	21.74	6.65	2.21	1.67	1.38	0.87	1.98	1.67
15	34.54	9.54	2.87	1.83	1.64	1.54	2.45	1.89
20	45.09	17.54	3.11	2.11	1.98	1.65	2.93	2.10
25	49.32	32.76	3.26	2.42	2.21	1.87	3.75	2.36
30	60.11	39.32	3.65	2.76	2.15	2.44	4.13	2.98
35	69.97	42.76	3.97	2.80	2.98	2.70	5.10	3.45
40	74.21	49.65	4.34	2.98	3.89	2.96	5.32	3.78
45	84.06	51.09	4.29	3.10	4.22	3.06	5.22	3.76
50	86.21	54.87	4.40	3.34	4.17	3.32	5.08	3.54
60	87.09	52.08	4.37	3.56	4.24	3.65	5.21	3.89
65	83.05	56.87	4.43	3.76	4.20	3.43	5.17	3.82

3.7. *HR*

Comparison of both control and experimental decomposition shows a gradual rise in concentration of HR (Table 3). On 10 days of decomposition control percentage of HR is rising as 1.67% and experimental as 2.21 % HR. At the end percentage of HR in both experimental and control is increasing constantly. Thirty days of decomposition of HR in control gives values as 2.76% HR and experimental gives 3.65% HR. If we compare the percentage of both control and experimental then the percentage of experimental is higher than the control value. On 30 days the comparative percentage of HR in both experimental and control is 24.38%.

3.8. *HI and DP*

On the 5 days of decomposition control and experimental HI gives value 1.40% and 1.3% respectively on the other hand control DP showing 0.90% and 1.1% experimental value (Table 3). On 40 days the concentration of HI and DP is rising constantly and gives values respectively 5.32% experimental HI, 3.78% control HI; similarly 3.89% experimental DP, 2.96% control DP. At the end of decomposition concentration of all increasing gradually on 65 days. Correspondingly, on 40 days of decomposition both experimental and control values gives percentage about 28.94% in HI 23.90% in DP. Comparison of both HI and DP percentages shows that HI gives more percentage value as compare to DP.

3.9. *Pearson correlation*

Composting is the decomposition of organic matter by microbial activity, which was enhanced by enrichment. It was found during composting the positive correlation was found between the humification parameters like HI, DP, HR and CEC. It means when the decomposition or humification of waste speedup then all the parameters showed the directly proportional to each other in their values (Table 4). In contrast the C/N ratio exhibited the decline with passage of time and negative correlation with other humification parameters. Present study results are in lined with various scientific publications [11,26-28].

Table 4. Pearson's correlation between chemical characteristics and maturity indices.

C/N	1	-0.90**	-0.92**	-0.94**	-0.91**	-0.90*
DP		1	0.97**	0.95**	0.98**	0.89**
HR			1	0.97*	0.94**	0.91**
HI				1	0.98**	0.95**
CEC					1	0.95**
Nitrate						1

* Correlation is significant at the 0.05 level (2-tailed). n.s: non-significant.
** Correlation is significant at the 0.01 level (2-tailed).

4. Conclusion

The addition of *Salmonella* in poultry litter and corn straw mixture for composting lessens the total carbon and C/N by increasing the process of decomposition which enhanced the pH of the compost and favored net ammonification and nitrification of composting mixture. It also enhanced the temperature, organic matter decomposition and ash contents accumulation. The compost stability and maturity was achieved during 40-45 days which was earlier in experimental trial than control. The use of such microorganisms isolated from parent waste can be further used on the road to the improvement of compost for different waste.

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