EVALUTION OF MICROBIAL ACTIVITY IN ORGANIC SOLID FRACTION DURING COMPOSTING PROCESS TO BIOFERTILIZER USING COMPOSTING BIN METHODS

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ABSTRACT

The current study aim to evaluating the composting in bins for organic fraction municipal solid waste and tracking the changes in physio-chemical and microbial characteristics such as Temperature, pH, C/N ratio and total viable count, total streptomyces, total coliform and pathogenic microorganisms as total *Salmonella Shigella* count. Seven treatments were used in research into the quick composting of the organic fraction of municipal solid waste. Two Composting bins contained municipal solid waste (MSW) mixed with animal and poultry manure (3:1), (2:1), waste mixture (2:1:1), and agricultural waste mixed waste. On the first day of composting, the pH was slightly acidic, then increased to alkaline, as EC . Microbiological parameters such as Total viable count are reduced during the thermophilic phase. They increased at the cooling phase , for TSC proliferation occurred in alkaline pH during composting and reached 4.1 CFU/g at maturation in composting bin 7. In conclusion, all composting bins developed a thermophilic phase, all had narrow alkaline pH with EC in some treatments less than 4mS.Cm^{-1}, and C/N reached the desired ratio as stable compost,also microbiological parameters fluctuate with temperature.

Key words: Physicochemical factors, total bacteria count, total coliform count, recycling

تقييم النشاط الميكروبي في الجزء العضوي من فضلات البلديه أثناء عملية تكوين السماد الحيوي باستخدام طريقة صندوق التسميد

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المستخلص

تهدف الدراسة الحالية تقييم انتاج السماد من الجزء العضوي في صناديق النفايات البلدية وتتبع التغيرات في الخصائص الكيموفيزيائيه والميكروبية مثل درجة الحرارة ودرجة الحموضة ونسبة C / N و التقييم الميكروبي من خلال التعداد الحي الكلي، مجموع البكتريا الخيطية، والكائنات الدقيقة القولونية الكلية والممرضة من خلال عد الاحياء المجهريه على وسط SS-Agar . تم استخدام سبعة معاملات في البحث للجزء العضوي من النفايات الصلبة البلدية. اثنين من صناديق التسميد يحتويان على نفايات صلبة بلدية (MSW) مختلطة مع روث الحيوانات والدواجن بنسبة (3: 1)، (2: 1)، خليط النفايات (2: 1: 1)، ومخلفات النفايات الزراعية المختلطة. كان الأس الهيدروجيني حمضيًا قليلاً في اليوم الأول من التسميد، ثم زاد إلى قلوي، وكذلك التوصيليه الكهربائيه. كما اكد ان النتائج من خلال التحاليل للسماد بان العد الكلي للبكتريا اختزل خلال الطور الحراري من عمليه التسميد. اعداد البكتريا الخيطيه الستربتومايسس, مع الزياده في الأس الهيدروجيني القلوي حيث وصلت إلى 4.1 وحده خلويه / جم في فتره التنضيج في معامله التسميد رقم 7. وبصوره عامه، جميع صناديق التسميد تكونت فيها الطور الحراي، وجميع المعاملات ذات قيم الأس الهيدروجيني القلوي مع التوصيليه الكهربائيه EC وصلت في بعض المعالجات (أقل من 4 ملي سيمنز.سم $^{-1}$)، كذلك نسبة C / N إلى النسبة المرغوبة كسماد ناضج. ولوحظ تغير في العوامل الميكروبيولوجية مع اختلاف درجات الحرارة، تم تثبيط نمو والتعداد الكلي للبكتريا القولونيه عند ارتفاع الحراره.

الكلمات المفتاحية: العوامل الفيزيوكيميائية، التعداد الكلي الحي للبكتريا, التعداد الكلي البكتريا القولونية، اعادة تدوير

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INTRODUTON

Climate change is a significant environmental concern, and waste disposal is the main contributor to severe ecological effects (25). Municipal solid waste (MSW) is the most complex waste stream; hence, its management system must be ecologically, economically, and socially acceptable (7,32). Composting is a management method for the reduction of solid waste load. Therefore, it may reduce the overall cost of solid waste disposal and provide a source of revenue by producing fertilizer at a low cost (32). The spontaneous biological breakdown of organic materials in a mostly aerobic environment; hence, composting has been extensively embraced as a waste recycling technology to recycle the organic portion of solid waste (1, 4). A successful biological process in several studies also cites that the composting process is the involvement of microorganisms in the decomposition of organic solid waste and temperature variations (3,5,22,25). Few research in Iraq has attempted to transform organic fractions of municipal solid waste into organic fertilizers throughout the years (18, 30). With an increase in municipal solid waste disposal in Iraq, recycling plants cannot accept the daily volume of waste from the populated area (31) Studies have demonstrated that composting solid organic waste decreases by more than 30% the amount of organic materials that wind up in already packed landfills or minimizes the cost of waste management via the generation of soil supplements (9,33). As shown by other researchers (35) composting on a small scale in bins is an effective way of assessing the physio-chemical and microbiological changes in the composting process, as well as the product fertilizers. Adopting the compost bins technique to recycle the trash in urban areas is sustainable and reduces waste transportation costs (21, 27). Another difficulty that has arisen is the appraisal of compost as a suitable mesocosm for *streptomyces* reproduction, which depends on the waste materials utilized in compost manufacturing. Gram-positive bacteria play a significant role in soil ecology, and hygienists of the produced fertilizer used indicator microorganisms such as coliform bacteria and pathogenic bacteria such as *Salmonella* and *Shigella*, particularly in waste mixture with organic amendments such as poultry and animal manure (10, 26). Many studies have focused on the influence of various organic waste mixtures and management strategies on the quality of final compost and the duration of the process (16, 24). Thus, the current study intends to evaluate composting in bins for organic fraction municipal solid waste and track the changes in chemical and microbial characteristics such as Temperature, pH, C/N ratio and total viable count, total *streptomyces*, total coliform and pathogenic microorganisms as total *Salmonella* and *Shigella* count.

MATERIALS AND METHODS

Study area: The current study was conducted in the garden of the College of Science for the Women / University of Baghdad from September to December 2020, using the aerobic bin composting technique at ambient temperatures between (20-39 °C). In contrast, the maturation process continues to the composts until April 2021. Composting bin technique scale was performed in plastic containers (0.4 × 0.4 × 0.90 m) with about six rows of 1.5 cm-diameter holes per side to enable aerobic decomposition. Inert materials (plastic, stones, glass….) were removed from MSW manually separated. After preparation, the mixtures for each treatment are manually turned twice a week during the first two weeks and then one time turned weekly afterwards. Microbiological activity is controlled under humidity of 40% -60%.

Experimental design: Seven experiments were designed in this study; The following treatments were utilized in this study:

Compost bin1: organic solid waste (control) 100%

Compost bin 2: organic solid waste: animal manure 3: 1 (75:25) (w/w) %

Compost bin 3: organic solid waste: poultry manure (chicken manure) 3: 1 (75:25) (w/w) %

Compost bin 4: organic solid waste: animal manure (2:1) (67:33) (w/w) %

Compost bin 5: organic solid waste: poultry manure (2:1) (67:33) (w/w) %

Compost bin 6: organic solid waste: agriculture waste 9: 1 (90:10) (w/w) %

Compost bin7: organic solid waste: animal manure: poultry manure (2:1:1) (50: 25: 25) (w/w) %

Compost materials consist of the organic part of municipal solid waste, animal, poultry (chicken) manure, and agricultural waste. All composting trials used organic fractions of municipal solid waste and animal, poultry, and agricultural waste as primary materials. The research included seven treatments comprised of organic fractions of urban solid waste combined with varying proportions of animal, poultry, and agricultural waste.

Physio-chemical analysis: The temperature was measured every day in the first four weeks and then once a week for the subsequent time using a thermometer with the aid of a metal probe inserted into 25-30 cm-deep holes. After manual turning, 500-gram samples at weeks 0,1,2,3,6,8,10,12,15, and 26 were from piles, and thereafter air dried in an open container, crushed to a 2 mm screen, and stored in polyethylene bags until laboratory analysis. Several physio-chemical parameters were examined, including: The pH and electrical conductivity (EC) of a water extract were determined by diluting one part of compost by volume with ten parts of distilled water at a ratio of 1:10 (w/v). Before being filtered using the process described by (28), the samples were shaken and allowed to precipitate. Organic matter (OM) percentage estimated by weight loss of samples after igniting at 550°C. Ten grams of composted samples were weighed, dried in an oven at 105 degrees Celsius for 24 hours, and then ignited at 550 degrees Celsius for four hours. The change in weight, referred to as Volatile compounds and C %, was computed using the calculations presented by Organic carbon (C%) = (OM%)/1.8 (39) Total nitrogen was measured using a modifed version of Kjeldahl's technique. Samples of compost were decomposed in concentrated H_2SO_4 (6).

Microbiological analysis

Fresh samples (500 grams) were gathered from surface and center of each compost bin at 0,1,2,3,6,8,10,12,15,26 week intervals and stored in the refrigerator for 72 hours prior to microbial culture. 5 grams of representative compost bin samples were weighed into 45 ml of 0.1 percent peptone and incubated at 37° C for 30 minutes. For the total viable count, total Coliform, pathogens *Salmonella*, and *Shigella*, total *Streptomyces* count, serial dilutions were performed from 10^{-1}- to 10^{-8}. On agar plates, 0.1 ml of supernatant from each dilution is transferred and cultivated using the spread technique. Nutritional agar was used to determine viable plate count at 37 °C for (24 – 48 hr). Total coliform on Mackonkey agar at 37 ° C (24hr), *Salmonella* and *Shigella* counts were determined on Salmonella Shigella agar after 18–24 hours at 36 °C, *Streptomyces* on Starch casein agar were used to cultivate a particular type of bacteria (7-16 days) . CFU/g was obtained by logarithmic of multiplying the number of colonies by the inverse of the dilution factor

RESULTS AND DISCUSSION

Composition of municipal solid waste: Recycling organic solid waste is vital for supplementing plant nutrients and increasing soil productivity. The presence of a significant amount of biodegradable more or less lies in part with the standard. Comparatively organic and inorganic materials in comparison to those of low nitrogen content were recorded (37). A total of 100 kg of municipal solid waste were taken randomly from the factory. After that, the composition of municipal solid waste was calculated as weight/weight as shown in the fractions of municipal solid wastes containing some reusable materials such as plastics, metals, paper, glasses and others, representing about 28.6%. This was separated and used for recycling for further uses. Vegetable matter and other decomposable were the predominant constituents which are present to the extent of 58 %, as shown in Table1. The results indicate that the organic fraction of municipal waste was highly suitable for composting. Similar observations have also been made by many earlier workers (19, 34, 38).

Table 1. The main composition of municipal solid waste understudy

No.	components	(%) by weight
1	Plastic	5
2	Metal	6
3	Paper	4.6
4	glass	6
5	Textile	4
6	Leather and rubber	3
7	Organic wate	58
8	Soil and stones	13
Total		99.6

Temperature: means of all treatments increased to thermophilic phase more than 45 ° C, with the exception of treatment 6, according to microbiological activity for the destruction of simple carbohydrates in the first phase, which is easily degradable material (17). The temperature rose in the third week. Composting bin 3 and 5 had a greater temperature range (57° C - 58 ° C) compared to composting bin 2 and 4, which had a lower temperature range (55° C - 56° C)(20). During development, the temperature of all compost bin treatments lowered progressively to that of the surrounding environment, as seen in the study of composting organic fraction of municipal solid waste (16). Composting bin 6 exhibited a longer mesophilic phase than the other treatments (Table 2). Although Composting bin 6 did not substantially vary from the control, this is not agreed with (18), which mixed vegetable waste with dry leaves to make better aeration for a longer thermophilic phase (6 days).

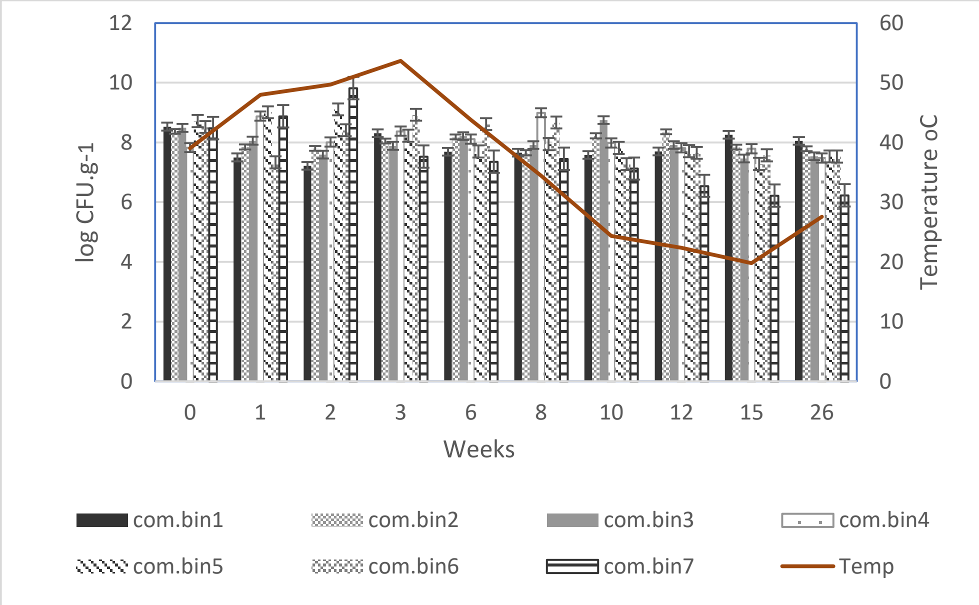
pH and electrical conductivity: At the beginning of waste composting, the pH ranges between 6.48 and 6.91, slightly acidic. In the mature phase, the pH tends to be alkaline, between 7.50 and 8.16, due to microbial respiration during the decomposition of organic matter and the release of ammonium. Due to buffering capacity and alkaline pH in the mature phase of composting, the pH did not vary much throughout the composting process (15). Electrical conductivity MSW combined with animal and poultry manure (2:1) had a higher EC value. During microbial degradation of added organic matter, the release of base cations (K+, Ca2+, Na+) and inorganic salts (phosphate and ammonium ions) may account for the high EC in these treatments agree with study (12) on sewage sludge . The accepted value for EC level in compost is less than 4 dS. m-1. For all treatments, the average was higher than the accepted level, but the final values (mature) were in level (Table 2).

C/N: During the composting process and maturation, the C/N ratio fell to acceptable values, indicating that the microbial biomass consumed the organic material. Initial C/N ratios were from 22.43 to 32.25, whereas maturity ratios ranged from 10.3 to 18.9, as predicted throughout the composting process (Table 2) . Compost bin 6 as mixed with dry leaves resulted in higher C/N due to a longer mesophilic period which disagrees with the results presented by (29). Their paper revealed that dry leaves are suitable for better aeration. Animal manure, combined with the organic component of solid waste with drate (3:1) and (2:1), resulted in the greatest decrease, as shown due to the longer thermophilic phase and its high nitrogen content concomitat with (2).

Table 2. Thermophilic Temperature (°C), C/N range and average physio-chemical properties(pH, EC) of composting bins

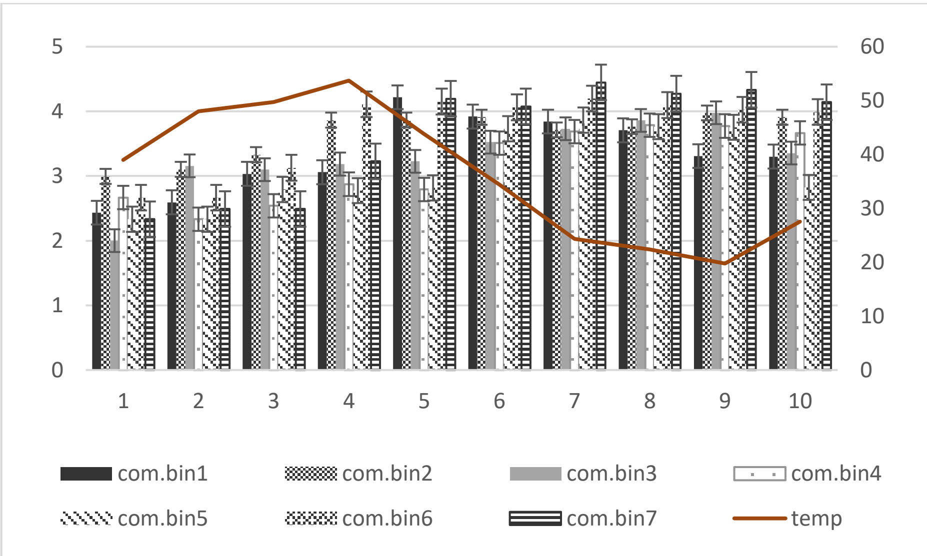
Compost bin	Temperature(C°) Thermophilic range	pH initial	pH final	EC Initial	EC final	C/N Initial	C/N final
1	47-49	6.7	8.05	3.8	3.04	29.6	13
2	44.5-57.0	6.48	7.50	3.78	3.65	29.42	12.04
3	43.5-57.5	6.7	7.65	4.11	3.66	24.61	11.9
4	44.5-55.5	6.49	8.40	4.05	4.33	32.3	10.27
5	45.5-58	6.53	7.78	4.26	5.1	27.24	16.80
6	44-45	6.9	8.16	4.21	4.32	28.71	18.9
7	44.5-55.2	6.85	7.85	4.10	3.83	22.4	11.4

A plate culture of successive dilutions on general and selective media depended on differences in the microbial population. The overall count at zero time was comparable at various mixing rates with animal manure. Different treatments begin the composting process with 8.3 to 8.7 pH for TVC (Figure 2a); composting bins 1,2,3,5 and 7 TVC decreased significantly at the end of the thermophilic phase, but composite bin 7 was higher in reduction TVC and reached 7.3 CFU/g in 6th week at the temperature at the end of the thermophilic phase. Compost bin 7 showed a significant decrease of (2.3) CFU compared to the control at the end of the composting process (P 0.05). In the third week, at 43.5°C, the CFU/g of compost bin 6 TVC grew to 8.9 CFU/g. In accordance with (36), the TVC for all piles after the end of the thermophilic phase fluctuates and decreases significantly at the mature stage. On average, there were no significant differences between treatments, but the seventh treatment had substantially less TVC than the other treatments. During the composting process, a load of total Coliforms, *Shigella*, and *Salmonella* were measured to determine the presence of pathogens in the compost. Up to 26 weeks, total coliform bacteria were detected in composite Bins 1 through 7. TCC in all composting bin treatments shows a considerable decline, while the number of colonies fell in composite bins 2,3,4 and 7 Figure (2c) during the thermophilic phase during the fourth week. According to (23), TCC may rise in specific treatments owing to recontamination by animal excrement or bird droppings, despite the fact that all treatments, with the exception of composting bin 6, show a considerable decrease in TCC compared to the control. Numerous works have determined the presence of bacterial pathogens, particularly Salmonella spp., during composting of different organic matter. Even though the time-temperature criteria were met, SS agar (salmonella shigella agar) was used as a counting medium for pathogenic bacteria, which decreased to less than 2 CFU/ g in composite bin 2, 3,4,5, and 7 in the thermophilic phase, while composting bin 1 and composite bin 6 resulted in fluctuations in ss agar count, this may account for frequent trash turns throughout the composting process, despite the fact that the composting bins 2, 3, and 4 were still within limits at the conclusion of the operation, this is consisted with (13) research on the composting of urban wastes and animal and poultry manure, that Faecal coliforms and Salmonella were wholly eliminated in all the compost systems after the 28th day with temperature values between 47°C – 60°C. The average number of Total Streptomyces count (TSC) in the waste mixture in the second composite bin was (3.6cfu/g), significantly higher than in the waste mixture in the third composite bin(3.3cfu/g). The average number of Streptomyces at the beginning waste mixture in the second composite bin was 2.9 CFU/g, while the waste mixture in the third composite bin decreased by 2 CFU/g. During the TSC composting process, temperatures were greatest at the compost bin's colder edges 2, 6, and 7, as shown in Figure (2b). Average TSC was lower in composting bin 5 even though the pH was alkaline, but temperature and EC higher than in other treatments. (Figure 2b). In our work, the total bacterial count (TVC) was raised throughout the cooling phase owing to the regrowth of spore-forming bacteria dropped during maturity. This result corresponded with (29) study on vegetable waste by composting bins. The first temperature rise was caused by the activity of mesophilic bacteria that are essential for the decomposition of organic substrate, which in turn causes a fast increase in temperature. This new situation supports the installation of the second co- composting mesophilic microflora (bacteria and actinomycetes) where Streptomyces proliferates in all composite bins once the temperature reaches 25Co - 32Co, but at the mature stage composting bin 5 were TSC may source of nitrogen and carbon limited to MSW and poultry manure that little bacterial diversity during composting (10). Total coliform is often employed as a measure of the overall hygienic condition of soil and water habitats. Utilizing an indicator, such as coliforms, as a substitute for the real disease-causing organisms is helpful since the indicators often appear more frequently than the pathogens and are safer to detect. Total fecal coliform (TFC) and salmonella shigella (pathogenic bacteria) were detected in municipal solid waste owing to human excrement diaper residue or non-sterilized clinical equipment. Pathogenic bacteria (SSagar) vanished during the thermophilic phase, despite its recovery during maturation in composting bin 5 as chicken manure employed in a 2:1 ratio as a source for a salmonella strain resistant to drought high temperatures (8).



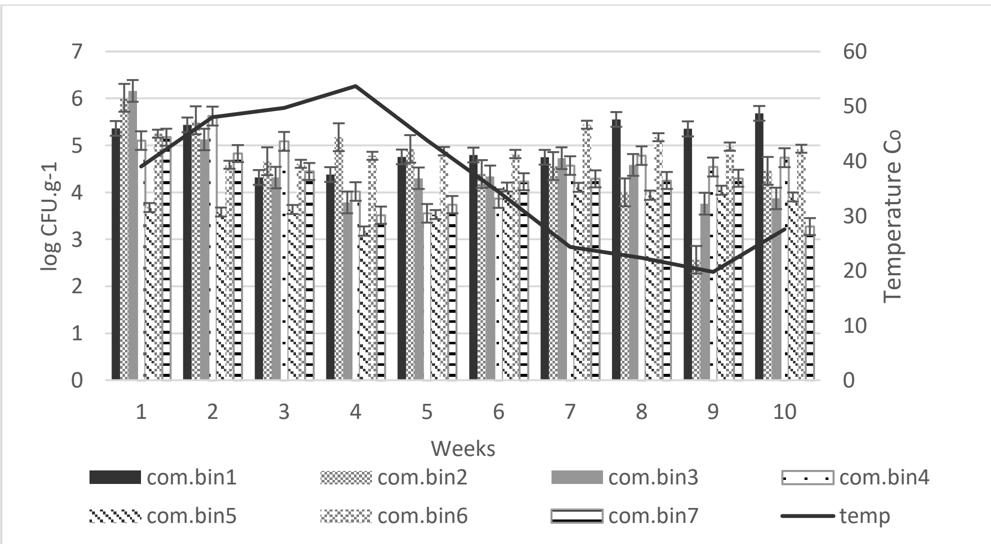
log CFU.g-1
Temperature oC
Weeks
0
1
2
3
6
8
10
12
15
26
com.bin1
com.bin2
com.bin3
com.bin4
com.bin5
com.bin6
com.bin7
Temp

2(a)



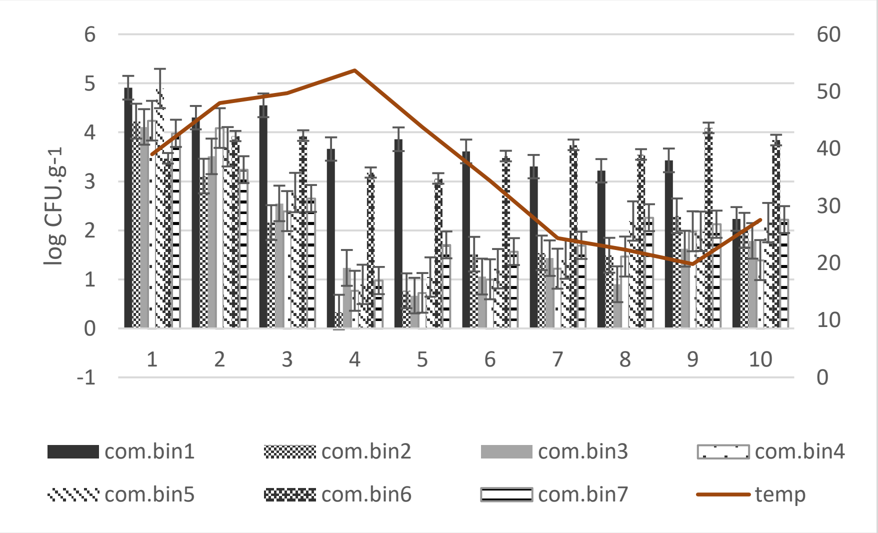
com.bin1
com.bin2
com.bin3
com.bin4
com.bin5
com.bin6
com.bin7
temp

2(b)



log CFU.g-1
Temperature Co
Weeks
com.bin1
com.bin2
com.bin3
com.bin4
com.bin5
com.bin6
com.bin7
temp

2(c)



2(d)

Figure 2. (a) represent TVC during composting period (b) Total *streptomyces* count (c) TCC (total coliform count) during composting (d) *salmonella – shigella* count

CONCLUSION

Increase EC value after maturation for composting bins included larger quantity of poultry (2:1). The total count of microbial groups changed substantially during composting. The total viable count reflects the influence of temperature fluctuations on indigenous bacteria, as seen in composite bins 3,5 and 7, which display very significant variations in the decrease of total viable count during maturation and organic matter breakdown . The majority of treatments exhibit a high *Streptomyces* count rise throughout the mesophilic period and rising in pH values. (Total coliform count) and Total *salmonella-Shigella* count (TSS) in bins 2, 3, and 7 demonstrate a substantial decrease in the thermophilic and mature phases . TSS was raised in some composting bines as a result of recontamination if they were not covered during maturation under sunlight.

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