Journal of Advances in Microbiology

6(3): 1-15, 2017; Article no.JAMB.36292
ISSN: 2456-7116

Activities of Locally Formulated and Commercial Effective Microorganisms in Composting of Organic Solid Wastes

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Authors' contributions

This work was carried out in collaboration between all authors. Authors GGE and UJJI designed the study, performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Authors OPA and BEND managed the analyses of the study. Author OPA managed the literature searches. All authors read and approved the final manuscript.

Article Information

DOI: 10.9734/JAMB/2017/36292
Editor(s):
(1) Jeyabalan Sangeetha, Department of Environmental Science, Central University of Kerala, India.
Reviewers:
(1) Mukhtar Ahmed, University of the Punjab, Pakistan.
(2) E. U. Eleanya, Veritas University, Nigeria.
Complete Peer review History: http://www.sciencedomain.org/review-history/21794

Original Research Article

Received 22nd August 2017
Accepted 16th October 2017
Published 7th November 2017

ABSTRACT

This study was carried out to evaluate the effectiveness of locally formulated Effective Microorganisms (EM) in the degradation of organic solid wastes. The study was laid out in a Completely Randomized Block Design with three replicates each. The research was conducted at the Centre for Genetic Engineering and Biotechnology, Federal University of Technology, Minna, Nigeria between May 2015 and May 2016. Microorganisms were isolated from waste dumps and were identified as bacteria (*Lactobacillus plantarum, Streptomyces griseus, Streptomyces rochei, Bacillus subtilis, Rhodopseudomonas palustris*), moulds (*Aspergillus oryzae, Aspergillus niger*) and yeast (*Saccharomyces cerevisiae*). The microorganisms were used to formulate Effective microorganisms (EM-A and EM-B), which was applied for biodegradation of organic solid wastes in compost. The activities of the two EM brands were compared with that of commercial effective microorganisms (EM-C) using physicochemical and microbiological parameters during the composting process. The compost treated with EM-A attained the highest temperature of 58ºC. The

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pH of all the treatments was alkaline at the end of composting, while moisture contents were below 50%. The total aerobic heterotrophic bacterial counts (TAHBC) showed that the highest count of 3.0 × 107cfu/g was recorded in P_1, Organic waste + EM-A on the 15th day of composting. The fungal counts varied among the treatments with the highest counts of 1.4 × 10^4 cfu/g recorded in P_3, Organic waste + Commercial EM (EM-C) on the 15th day. Comparing the fertilizer value of compost obtained from the different treatments using their chemical properties, OF_1 (Organic fertilizers with EM-A), had the best fertilizer value based on the N-P-K values (2.48 g/kg, 2.48 mg/kg and 2.51 cmol/kg respectively) and therefore, is most suitable for crop use. The activities of Effective microorganisms were responsible for the significant differences in physicochemical and microbiological parameters.

Keywords: Effective microorganisms; composting; temperature; organic waste.

1. INTRODUCTION

The abundance of municipal solid waste (MSW) has become a global issue especially in developing countries. The progress of modern civilization and technological advancement, rise in population with increased migration to urban areas, changes in life style and consumption pattern, have all contributed significantly to the increase in quality and variety of waste generated [1,2]. Organic wastes constitute between 45% and 65% of the municipal solid wastes [2,3,4,5]. Naturally, microorganisms degrade these wastes at different rates in the natural environment but the biodegradability of the organic waste material depends on the nature of the material and the characteristics of the environment where the material is. To create an enabling environment for the quicker biodegradation of these wastes, the wastes are subjected to composting. Mridha [6] defined composting as a process of biological decomposition of organic wastes which is carried out by a group of active microorganisms that break down the cellulolytic materials and hasten the process under aerobic condition at an elevated temperature. Composting has been an old practice even in the local settings but the speed at which waste decomposition takes place can be enhanced to an appreciable level by controlling certain environmental factors such as temperature, pH, moisture content, carbon- to- Nitrogen (C:N) ratio, particle size [7,8].

The activities of the indigenous microorganisms in the waste materials in compost can also be enhanced by the use of additional microorganisms termed Effective Microorganisms (EM) [9]. EM is a combination of useful regenerated microorganisms that exist freely in nature and is not manipulated in any way [10]. They are known to be effective, disease-suppressing microorganisms which work in synergy to speed up biological processes. Five groups of microorganisms make up the effective microorganisms as follows: Lactic acid bacteria, yeasts, Actinomycetes, photosynthetic bacteria and fermenting fungi [11,12]. Lactic acid bacteria in its mutual relationship with other microbial members of effective microorganisms produce lactic acid which is a strong sterilizing compound [13]. As members of effective microorganisms, yeasts synthesize antimicrobial and other useful substances required for plant growth from amino acids and sugars secreted by photosynthetic bacteria, organic matter and plant roots. The bioactive substances such as hormones and enzymes produced by yeasts promote active cell and root division. These secretions are also useful substrates for effective microbes such as lactic acid bacteria and actinomycetes [13].

Actinomycetes suppress harmful fungi and bacteria and can live together with photosynthetic bacteria. Photosynthetic bacteria play the leading role in the activity of effective microorganisms. They synthesize useful substances from secretions of roots, organic matter and/or harmful gases (e.g. hydrogen sulphide) by using sunlight and the heat from the soil as sources of energy [13]. These bring about fermentation that breaks down the organic substances quickly to produce alcohol, esters and antimicrobial substances. Fungi suppress odours and prevent damage that could be caused by harmful insects and maggots [13]. The objective of this study is to evaluate the effectiveness of locally formulated Effective Microorganisms (EM) in the degradation of organic solid wastes.

2. MATERIALS AND METHODS

2.1 Collection of Samples

Soil samples were collected at 6 cm-20 cm depth from different points at waste dump sites in Minna metropolis, Niger State, Nigeria into clean polythene bags and transported to the laboratory for the isolation of microorganisms. Spoilt oranges were collected from the waste dump sites in Minna for the isolation of yeasts.

Organic solid wastes were collected in waste collection bags (polythene) of 50 kg capacity from households and restaurants in Minna, Niger State, Nigeria. The waste materials were manually sorted out thoroughly to remove metals, plastics, stones, rags, glasses, broken bottles. The organic waste materials left for use in composting were mainly vegetables, fruits, dried grasses, food wastes, dried and green leaves, weeds, twigs, corn cobs, stalks. The sorted waste materials were shredded into small sizes and were mixed thoroughly for layering into the compost bin. Commercial EM was obtained from Macmed Integrated Farms, Lagos, Nigeria.

2.2 Isolation of Microorganisms

The soil sample was serially diluted and 1 ml of 10^6 of the suspension was inoculated on the various media (Yeast-extract peptone dextrose agar, YPDA (distilled water 1 L, dextrose 20 g, peptone 10 g, yeast extract 5 g and agar 20 g); Man Rogosa sharpe agar, MRS – Titan Biotech Limited, CAT- TM 146; Nutrient agar, NA – SRL Pvt Limited, CAT- NM 011; Starch casein agar, SCA (Starch 10.0 g, Casein 0.3 g, KNO_3 2.0 g, NaCl 2.0 g, K_2HPO_4 2.0 g, $MgSO_4$ $7H_2O$ 0.05 g, $CaSO_4$ 0.02 g, $FeSO_4$ 0.01 g, Agar 20.0 g, distilled water 1L) and Sabouraud dextrose agar, SDA – SRL Limited, CAT- SM 011). The inoculated YPDA, SDA and SCA plates were incubated at 30ºC for 48hours to isolate yeasts; 3days to isolate *Aspergillus* species and 5 days to isolate *Streptomyces* respectively [14,15]. Inoculated MRS agar plates were incubated at 37ºC in an anaerobic jar for 3 days to isolate lactic acid bacteria [16]. Nutrient agar (NA) plates were incubated at 30ºC in the dark for 7 days to isolate photosynthetic bacteria and another at 37ºC for 24 hours to isolate non-photosynthetic bacteria.

2.3 Characterization and Identification of Microbial Isolates

2.3.1 Bacterial isolates

The isolates were characterized based on their cultural and morphological characteristics as well as biochemical tests which include the production of catalase, oxidase, indole, urease,

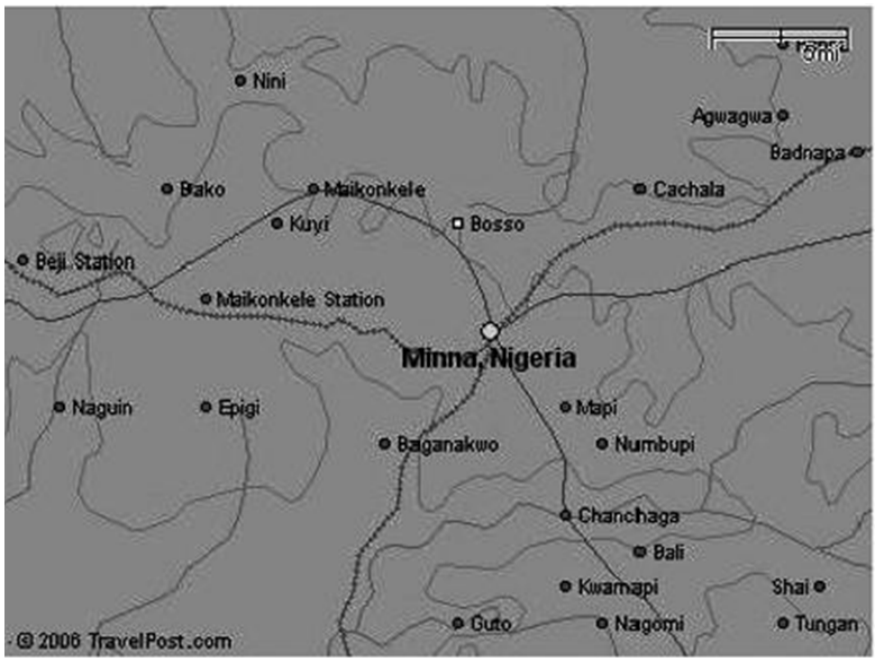

Fig. 1. Map of Minna, Niger State, Nigeria

methyl red and Voges Proskauer test, Citrate utilization test. Gram staining and the biochemical tests were carried out according to methods outlined by [17]. The identities of the isolates were confirmed by comparing their characteristics with those of known taxa using Bergey's Manual of Determinative Bacteriology [18]. Molecular ribotyping of the bacterial isolates was carried out using the partial gene sequence of 16S rDNA in order to further confirm the identities of the isolates. PCR ingredients used were Deionized water, lysis buffer, dNTP, Forward and Reverse primers, template DNA. 100bp ladder and 1kb DNA ladder were used as molecular size markers.

2.3.2 Mould isolates

Mould isolates were characterized based on the colour of aerial and substrate mycelium, nature of hyphae, shape and kind of asexual spore, appearance and characteristics of spore head. A wet mount was prepared by placing a drop of Lactophenol cotton blue and observed under the microscope with x10 and x40 objectives. The identification was carried out using the scheme of [19] and [20].

2.3.3 Yeast isolates

Yeast isolates were characterized based on morphological, biochemical and physiological tests which include the ability to ferment sugars, ability to grow at different temperatures and concentration of ethanol [21]. The identification was carried out using the scheme of [22].

2.4 Screening of Microbial Isolates for Cellulose Utilization

Microbial isolates were streaked on cellulose Congo-red agar media with the following composition : KH_2PO_4 0.5 g, $MgSO_4$ 0.25 g, cellulose powder 2 g, agar 15 g, Congo-red 0.2 g Gelatin 2 g, distilled water 1 L and at a pH 7.0. The plates were inoculated with the isolates and incubated at 30ºC for *Aspergillus* and *Streptomyces* and at 37ºC for bacteria and yeast until substantial growths were observed. The colonies which showed discolouration of Congo-red were regarded as positive cellulose-degrading organisms [23].

2.5 Formulation of Effective Microorganisms (EM)

The isolated microorganisms were cultured together in 3% molasses at pH 7.0 and were incubated at 37ºC for 3 days to form EM [24].

2.6 Weighing of Samples

Each empty composting bin was first weighed and the weight recorded as W_0. After filling the bin with organic waste materials, the new weight was taken as total weight (W_1). The weight of the waste sample (W_2) was determined using the formula:

Weight (Kg) of the sample (W_2) = Total weight (W_1) – Weight of empty composting bin (W_0).

2.7 Activation and Application of Effective Microorganism (EM)

Effective microorganism, EM-A and EM-B were applied as originally formulated while EM-C (commercial EM) was activated using water and molasses according to [25]. A layer of 10 cm of organic waste materials was made in the compost bin and 150 ml of activated effective microorganism was sprayed on it and this was repeated after every layer until the bin was filled.

2.8 Compost Preparation

The experiment was set up in Completely Randomized Block Design with three replicates and this includedP_1: Organic waste materials + EM-A; P2: Organic waste materials + EM-B; P3: Organic waste materials + Commercial EM (EM-C); P_4: Organic waste materials (uninoculated) as a control. A basket of 34 cm height and 34 cm width was used as the compost bin, which was set up in an open shade to keep away direct rain and sunlight.

2.8.1 Monitoring of the compost process

The compost was turned every three days for aeration throughout the composting period of three weeks and was moisturized by sprinkling water whenever necessary. Samples of the compost were collected from different points of the heaps and were mixed thoroughly for microbiological and physicochemical analyses.

2.8.2 Microbiological analysis

Fifty grams (50 g) of the compost materials was dispensed in 950 ml of sterile distilled water and subjected to shaking for 2 h in order to dislodge the microorganisms from the waste materials.

This was serially diluted to 10^6 and 1 mL was pour plated on Nutrient agar and incubated at 37ºC for 24-48 h for the enumeration of total aerobic heterotrophic bacteria; and on SDA, incubated at 28±2ºC for 3-5 days for the enumeration of fungi. The colonies which developed after incubation were recorded as colony forming units per gram (cfu/g) of the compost material.

2.8.3 Physicochemical analysis

The temperature, pH and moisture contents of the composts were determined to assess the changes in the compost according to [3,26].

2.8.4 Determination of compost maturity

The outcome of the physical (colour, smell, appearance) and chemical parameters of the compost which were monitored was used to confirm the stability of the compost.

2.9 Determination of the Chemical Properties of Organic Fertilizers

The chemical properties of the organic fertilizers which included pH, Organic matter, Organic carbon, Total Nitrogen, Available phosphorus, potassium, sodium, calcium and magnesium were determined using standard methods according to Gautam et al. [27].

2.10 Statistical Analysis

All data generated were subjected to statistical analysis using the Analysis of variance (ANOVA). The Least Significant Differences (LSD) in the effects of variable treatments was considered at 5% Probability level ($P< 0.05$).

3. RESULTS AND DISCUSSION

3.1 Identification of Microbial Isolates in Waste Dump

3.1.1 Bacteria

Sixteen bacteria were isolated from waste dump but based on their relevance in the present study, five were identified as *Lactobacillus plantarum, Streptomyces griseus, Streptomyces rochei, Bacillus subtilis and Rhodopseudomonas palustris*. The *L. plantarum* isolated in this study using de Man Rogosa and Sharpe (MRS) agar medium, showed spottish white growth after 72 h incubation in an anaerobic jar at 37ºC. The isolate was Gram positive, catalase negative rods that could not grow at temperature of 45ºC. *Rhodopseudomonas palustris* was isolated from waste dump soil near water bodies. After incubation on nutrient agar at 30ºC for 5-7 days in the dark, there were growths of orange colonies as described by [28]. The Gram reaction showed Gram negative rods. Biochemical tests which included citrate, catalase, mannitol, sucrose, starch hydrolysis were carried out and in the report of [29], the criteria used to validate the bacterial isolate as *R. palustris* agree with results of this study.

In the present study, the *Bacillus* species isolated from soil was identified as *B. subtilis* using Gram reaction, morphological, characteristics and biochemical tests. Actinomycetes which included *Streptomyces griseus* and *Streptomyces rochei* isolated in this study showed differences in their cultural characteristics. While *S. griseus* showed chalky white colonies, *Streptomyces rochei* showed gray white colonies which turned the starch casein agar medium brownish. Both species were Gram positive rods but showed differences in the utilization of sucrose. While *S. griseus* was sucrose positive, *Streptomyces rochei* was sucrose negative. Their reaction to catalase and indole were also different.

The DNA extracted from the bacterial isolates were subjected to PCR and was amplified for identification of the species. The amplified DNA products were confirmed through Gel Electrophoresis and by visualization of their band patterns. Based on the 16S rRNA, the bacteria were confirmed as *Rhodopseudomonas palustris, Bacillus subtilis* and *Lactobacillus plantarum,*

3.1.2 Yeasts

Two strains of *Saccharomyces cerevisiae* were isolated from waste dump. One of the two strains Sc-1 was isolated from decayed orange fruit from the waste dump while the other Sc- 2 was isolated from the soil from the waste dump. The strain Sc-1 showed white, opaque, smooth and spherical colonies while the strain Sc- 2 showed creamy, smooth and spherical colonies. All the morphological features of the isolates corresponded to *S. cerevisiae* [30]. The differences in biochemical reactions showed that Sc-1 was able to utilize mannitol while Sc-2 was mannitol negative. The presence of this yeast in rotten fruits could be an indication that it plays a role in waste degradation. This could as well be the reason why Patel and Rajkumar [31] isolated *S.cerevisiae* from soil samples collected from a landfill site.

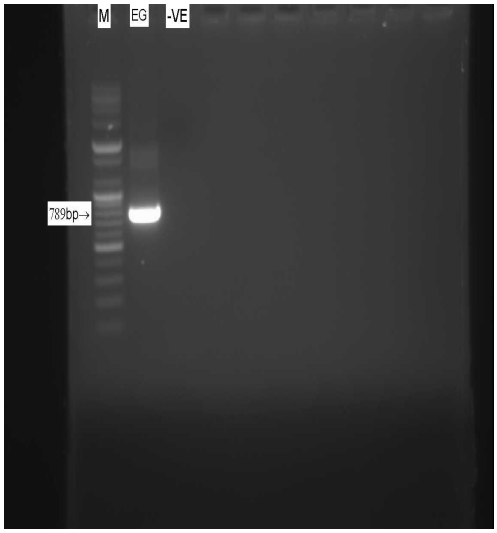

Fig. 2. The PCR amplicon of 16S rRNA gene obtained from *Rhodopseudomonas palustris*
Lane M: Lamda DNA double digest marker
Lane EG: Amplified 16S rRNA gene

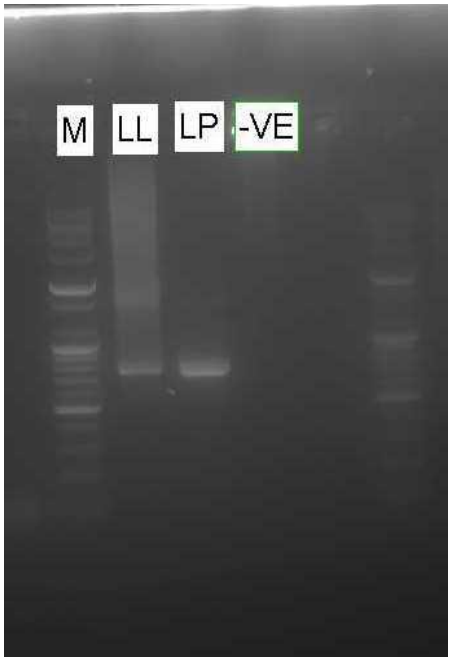

Fig. 3. The PCR amplicon of 16S rRNA gene obtained from *Bacillus subtilis* and *Lactobacillus plantarum*
Lane M: Lamda DNA double digest marker
Lane LL: Amplified 16S rRNA gene Bacillus subtilis.
Lane LP: Amplified 16S rRNA gene Lactobacillus plantarum.
Lane –VE: Negative control

3.1.3 Moulds

The fungi identified from waste dump were *Aspergillus oryzae* and *Aspergillus niger*. While *A. oryzae* showed greenish yellow and brownish yellow colours of aerial and substrate hyphae respectively on SDA, *A. niger* showed black and brown colours respectively. The macroscopic characteristics of the isolate when compared with a reference *A. oryzae* used by [32] was identical. The microscopic characteristics were also similar to that of the reference with respect to conidia, vesicles and mycelia. Elbashiti et al. [32] isolated *Aspergillus oryzae* from contaminated rice, soybean and wheat. Sooriyamoorty et al. [33] and [34] also isolated *A. oryzae* from food materials. This could be the reason why the microorganism, *A. oryzae* was isolated from waste dump soil in this study, since contaminated foods are eventually disposed of at the waste dump.

3.2 Cellulose Utilization by Microbial Isolates

The microbial isolates that were able to utilize cellulose were: *Bacillus subtilis, Aspergillus oryzae, Aspergillus niger, Streptomyces griseus* and *Sreptomyces rochei*. In the study of [35], the ability of *Bacillus subtilis* to utilize cellulose activity was enhanced by optimizing the medium with CMC, peptone and yeast extract using Placket- Burman design. Simlarly *A. niger* exhibited the ability to degrade cellulose by its cellulase production activity. Acharya et al. [36] reported the production of cellulase by *Aspergillus niger*, for improvement of enzymatic hydrolysis of saw dust.

Ram [37] reported that *Streptomyces griseus* did not produce the enzyme cellulase. However, in the present study, *Streptomyces griseus* showed a wide zone of clearing (60 mm) on cellulose agar plate when it was flooded with 1% Congo red. *Aspergillus oryzae* demonstrated cellulose activity on cellulose Congo red agar plates. A zone of clearing of 30mm around the colonies was used as an indication for cellulose utilization. The use of Congo red as an indicator for cellulose degradation in an agar medium provides the basis for a rapid and sensitive screening test for cellulolytic bacteria [26]. The formation of clearing zone around the colonies confirms the secretion of extracellular cellulose.

3.3 Effective Microorganisms Formulations

The colony counts (cfu/ml) of microorganisms that constituted the effective microorganisms (EM-A and EM-B) are as follows: *Lactobacillus plantarum* 1.0×10^8, *Saccharomyces cerevisiae* 2.0×10^6, *Aspergillus oryzae* 1.2×10^5, *Streptomyces griseus* 3.0×10^5, *Rhodopseudomonas palustris* 1.3×10^5 for EM-A. The microorganisms that constituted EM-B were *Bacillus subtilis* 1.4×10^5, *Streptomyces rochei* 1.3×10^5, *Saccharomyces cerevisiae* 2.1×10^3, *Aspergillus niger* 1.0×10^5. These microorganisms were mixed with 3% molasses and incubated at 37ºC for 3 days. At the end of the incubation period, the pH of the consortium reduced from 7.0 to 3.8.

3.4 Weight of Organic Waste Materials Used for Composting

Each compost bin was filled with solid wastes of weight of 10kg at the start of composting. It was observed that there was a gradual decrease in the weight of the compost as the composting process progressed with about 70-80% loss in weight of waste at the end of composting (after three weeks) in treatments P_1 (Organic waste materials + EM-A), P_2 (Organic waste materials + EM-B), P_3 (Organic waste materials + Commercial EM) and 70% loss in weight of wastes in P_4 (Organic waste materials) (Table 1). The reduction in weight of wastes in this study is higher than that recorded by [38], who reported a maximum weight reduction of about 65%.However, the result of weight loss is in agreement with the report of [27], who recorded more than 70% weight loss in composting of municipal solid waste (MSW) during summer season. The reduction in weight could be as a result of biochemical breakdown to about 30 to 50 percent of what went into the waste pile. The presence of much woody materials remaining in the treatment, P_4 after the composting period could have resulted in a higher weight than other treatments.

3.5 Microbial Counts

The total aerobic heterotrophic bacterial counts (TAHBC) varied during the composting process. At the beginning of composting, the TAHBC were 10.0×10^6 cfu/g for P_2 (Organic waste materials + EM-B) and 18.0×10^6 cfu/g for P_3 (Organic waste materials + Commercial EM). The counts increased as composting progressed as P_1 (Organic waste materials + EM-A) and P_2 had counts that rose from 15.0×10^6 cfu/g on day zero to 24.5×10^6 cfu/g and from 10.0×10^6 cfu/g to 25.0×10^6 cfu/g respectively on the sixth day. P_3 and P_4 (uninoculated) recorded a decrease in TAHBC from 18.0×10^6cfu/g on day zero to 9.3×10^6 cfu/g and from 17.6×10^6 cfu/g to11.0×10^6 cfu/g respectively on the sixth day (Fig. 4). There were significant differences ($P< 0.05$) in the counts obtained in the various treatments. The high counts recorded initially in all treatments may be because bacteria are the most numerous microorganisms in compost and make up 80% to 90% of the billions of microorganisms found in a gramme of compost [39]. Trautmann and Olynicw [40] reported that compost piles have billions of microbes, mostly bacteria because they love the conditions of air, moist and heat. The application of effective microorganisms into the compost could have also caused a boost of the microbial counts. The increase in temperature above 40ºC which was recorded from the 4^{th} day of composting could have caused a decrease in the bacterial counts in P_3. However, the changes in bacterial counts were unstable but increased uniformly on day 15 and day 18, particularly P_1, P_2 and P_3. All increases or decreases in bacterial counts in all the treatments could be due to changes in the nutritional, environmental and operational conditions of the compost [41].

Fungal counts were erratic for all treatments. The counts in P_1 ranged from $3.0\times10^6$to 21.0×10^6cfu/g, $2.0\times10^6 - 11.0\times10^6$ cfu/g for P_2, $2.0\times10^6 - 15.0\times10^6$ cfu/g for P_3 and $0 - 12.0\times10^6$ cfu/g for P_4 (Fig. 5). There were significant differences ($P< 0.05$) in the counts obtained in the various treatments. The change in pH from acidic to alkaline in the treatments could have caused the variations in fungal counts since fungi prefer acidic growth conditions [42]. The fungal counts decreased in all treatments as the temperature rose above 40ºC on the sixth day except for P_4. This could be so since fungi prefer cooler temperatures (20ºC - 25ºC) although some can survive high temperatures up to 45 - 55ºC [43]. The erratic nature of fungal counts in this study could be due to some environmental factors such as availability of nutrients, the presence of antibiotics secreted by other microorganisms, which can ward off other competing fungi, high temperatures and depletion of primary food sources [44].

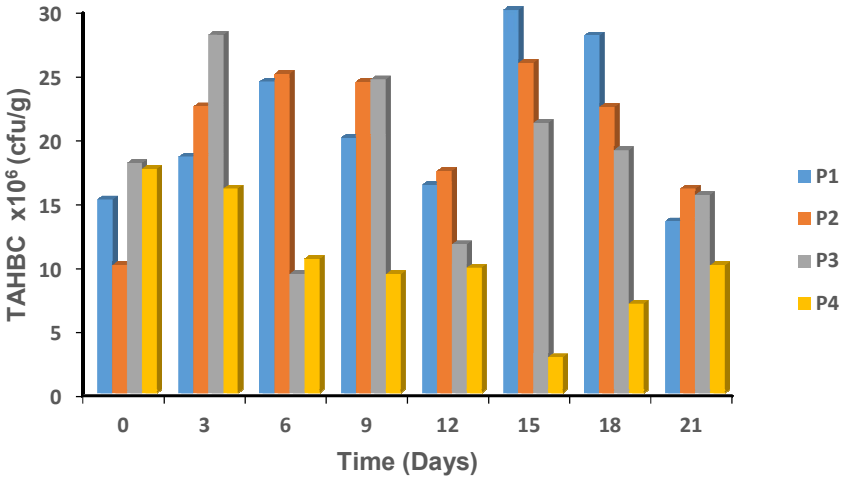

Fig. 4. Total aerobic heterotrophic bacterial counts (TAHBC) in compost produced using effective microorganisms

P_1: Organic waste + EM A, P_2:Organic waste + EM B, P3: Organic waste + Commercial EM (EM C), P4: Organic waste (uninoculated)

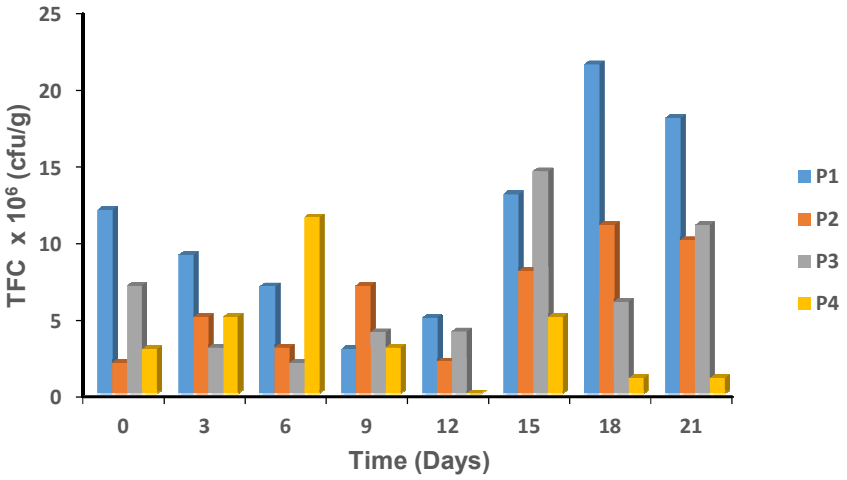

Fig. 5. Total fungal counts (TFC) in compost produced using effective microorganisms

P_1: Organic waste + EM A, P_2: Organic waste + EM B, P_3: Organic waste + Commercial EM (EM C), P_4: Organic waste (uninoculated)

3.6 Physicochemical Properties of Waste Materials in Compost

3.6.1 Temperature

The temperature of the compost at zero day of composting ranged between 20ºC in P_2 (Organic waste + EM-B) and 23ºC in P_1 (Organic waste + EM-A). The temperature followed a regular increase within the first 6 days of composting, rising up to 56ºC in P_2 on the 6th day. The increase in temperature lasted till the 15th day in all treatments. The initial rise in temperature might have been caused by the initial decomposition of the compost waste materials by mesophilic microorganisms, which rapidly breakdown the soluble, readily degradable compounds, producing heat which causes the compost temperature to rapidly rise [40,45]. The temperature of the compost exceeded the mesophilic phase and rose above 40ºC into the thermophilic phase after the third day except P_3

(Organic waste + Commercial EM, EM C) which rose to 44ºC on the 3rd day. The report of [27] recorded a temperature rise to 48ºC after one day of composting, which is in contrast to the result of the present study.

P_1 attained 58ºC on the 9th and 15th day and this was the highest temperature recorded throughout the composting period in all treatments. This temperature pattern is similar to that of P_2 and could be as a result of turning of the compost. If the compost pile is turned after the thermophilic stage, it will heat up again and this cycle can repeat 3 - 4 times until the energy supply for the thermophiles is exhausted [3]. After the fifteenth day, there was no rise in temperature in all the treatments. However, there were significant differences ($P< 0.05$) in the temperatures of the treatments. The highest temperature recorded in P_4 (Organic waste (uninoculated)) was 50ºC. The rise in temperature is a measure of the heat generated in microbial metabolism and the effectiveness of the thermal insulation provided by the compost mass and by any cover or container enclosing the mass. Hence, the activities of Effective Microorganisms (EM) may have affected the temperatures attained by each treatment in this study. The thermophilic phase lasted for six to eight days in P_1, P_2 and P_3 and this was enough periods for elimination of pathogens in the compost [46] while it lasted for only three days in P_4. Adewumi et al. [3] also reported that high temperatures above 55ºC for 6 – 8 days can destroy most pathogenic microorganisms and eggs of common intestinal worms where human faecal wastes are used in composting. This implies that, from the results of this study, treatments P_1, P_2 and P_3, which were inoculated with EM, can be assumed to be free of pathogens. The duration of the active phase depends on the characteristics of the waste (amount of easily decomposable substances) and the management of the controlling parameters (aeration and watering) [47]. As the temperature decreased gradually after the 15th day, there was resetting of the mesophilic phase, causing bacterial and fungal counts to increase gradually. The temperature remained low in spite of moistening and turning of the compost. This could be due to the fact that microbial activities were less intense after the readily decomposable components had been degraded and only the more refractory components were remaining. The temperature pattern of all the treatments had almost the same stabilization progress. After the 15th day, the volume of the organic wastes had obviously reduced and the temperature changes had become minimal with little or no changes in temperature observed on the 21st day (Fig. 6). It has been reported that the compost mass can be safely used or stored after the temperature had finally dropped to about 40ºC.

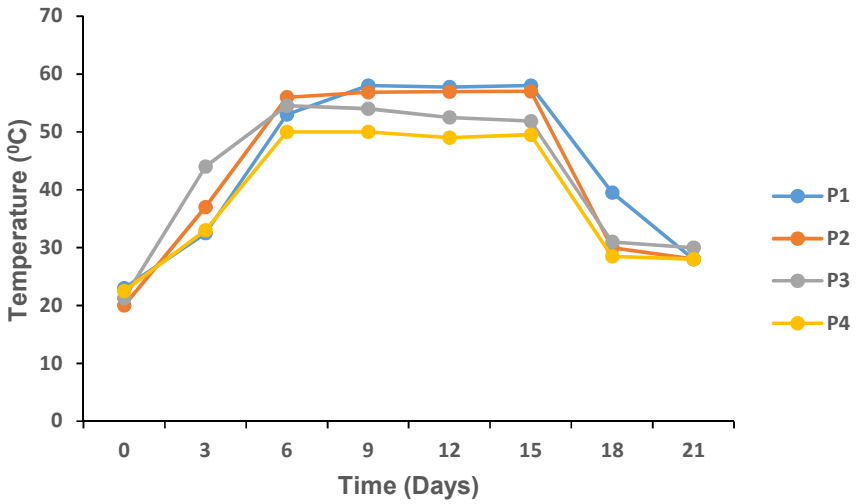

Fig. 6. Temperature (ºC) of the compost produced using effective microorganisms (EM)
P_1: Organic waste + EM A, P_2: Organic waste + EM B, P_3: Organic waste + Commercial EM (EM C), P_4: Organic waste (uninoculated)

3.6.2 pH

The initial pHs in all treatments were acidic ranging from 6.4 to 6.5. P_2 and P_3which are organic waste + EM B and organic waste + Commercial EM (EM-C) turned alkaline with pH 8.1 and 8.0 respectively on the sixth day. On the 9^{th} day, the pH of all treatments had become alkaline ranging from 7.7 in P_1 to 8.9 in P_2 (Table 2). The acidic pH recorded in the first 3 days of the study might be due to the initial degradation of food wastes leading to the production of organic acids [46]. The pH values fluctuated throughout the composting period in all treatments with the lowest pH of 5.8 recorded in P_1 (Organic waste + EM A) on day 3 and highest pH of 8.9 recorded in P_2 on day 9. The control (P_4), recorded mainly acidic pH initially but tended to neutrality and slightly alkaline at the end of the process, probably because the organic wastes were attacked by the microorganisms at a slow rate. There were significant differences ($P< 0.05$) in the pH values of the treatments. Gomen-Brandon [45] suggested that the organic acids produced at the initial decomposition served as substrates for succeeding microbial populations. The subsequent rise in pH therefore reflects the utilization of the acids by the microbes. The alkaline pH may be due to accumulation of ammonia (NH_3) released during the decomposition of Nitrogen-containing organic matter. The NH_3 dissolves in the moisture present to form alkaline NH^{4+} [46,48]. According to [49], the optimal pH range is 6.0 - 7.5 for bacteria and 5.5 - 6.0 for fungi. In this study however, the counts of bacteria and fungi cut across pH values up to 8.0. The wide variation and higher pH recorded in this study could be as a result of the composition of the feedstock, which researchers including [50] reported to play a role in determining the pH of compost.

3.6.3 Moisture

The moisture content was high (60 - 80%) on day zero of composting for all treatments. It however decreased to 54 - 60% for all treatments after 3 days and rose to between 64% and 69% in P_1-P_4 on the sixth day (Fig. 7). The initial high moisture content might be due to the original feedstock of the compost which contained food waste and other kitchen wastes [46]. The decrease on the other hand, after 3 days, could be due to escape of moisture to the atmosphere from the compost. The second rise no doubt was due to moistening of the compost materials. From the ninth day, it was observed that the moisture level decreased steadily in all treatments though at different rates. The steady decrease could be due to the fact that the amount of water used to moisten the compost was reduced subsequently, throughout the period of composting. The moisture levels of all the treatments were below 50% at the end of the composting process after 21 days. There were significant differences ($P< 0.05$) in the moisture levels of all the treatments. The results of this study agree with the report of 56) in which the final moisture of compost was between 45% and 50%. US Composting Council [51] also reported that the preferred moisture content of finished compost should be between 40 and 50%.

3.7 Physical and Chemical Properties of Organic Fertilizers

The physical properties of the organic fertilizers produced which include the colour, earthy smell [52], the absence of recognizable components of the waste materials as well as the monitored chemical parameters confirmed the stability of the compost. The dark-brown colour of the organic fertilizer is in agreement with the report of [1] and [53]. It was observed that the organic

Table 1. pH of compost produced using effective microorganisms

Time (days)	P_1	P_2	P_3	P_4
0	6.45 ± 0.05^{a}	6.50 ± 0.00^{a}	6.40 ± 0.00^{a}	6.45 ± 0.05^{a}
3	5.85 ± 0.05^{a}	6.85 ± 0.05^{c}	6.55 ± 0.05^{b}	6.85 ± 0.05^{c}
6	6.05 ± 0.05^{a}	8.10 ± 0.10^{d}	8.00 ± 0.00^{d}	6.60 ± 0.00^{b}
9	7.75 ± 0.05^{b}	8.95 ± 0.05^{e}	8.05 ± 0.05^{c}	8.65 ± 0.05^{d}
12	6.95 ± 0.05^{b}	6.00 ± 0.00^{a}	7.65 ± 0.05^{c}	7.05 ± 0.05^{b}
15	7.50 ± 0.00^{b}	7.65 ± 0.05^{b}	8.00 ± 0.00^{c}	7.10 ± 0.10^{a}
18	7.95 ± 0.05^{d}	7.55 ± 0.05^{b}	7.70 ± 0.00^{c}	7.50 ± 0.05^{b}
21	8.00 ± 0.00^{d}	7.70 ± 0.00^{c}	7.75 ± 0.05^{c}	7.40 ± 0.00^{b}

^{a,b,c,d,e}: means denoted by different superscripts along the same row are significantly ($p<0.05$) different.P_1: Organic waste + EM A, P_2: Organic waste + EM B, P_3: Organic waste + Commercial EM (EM C), P_4: Organic waste (uninoculated)

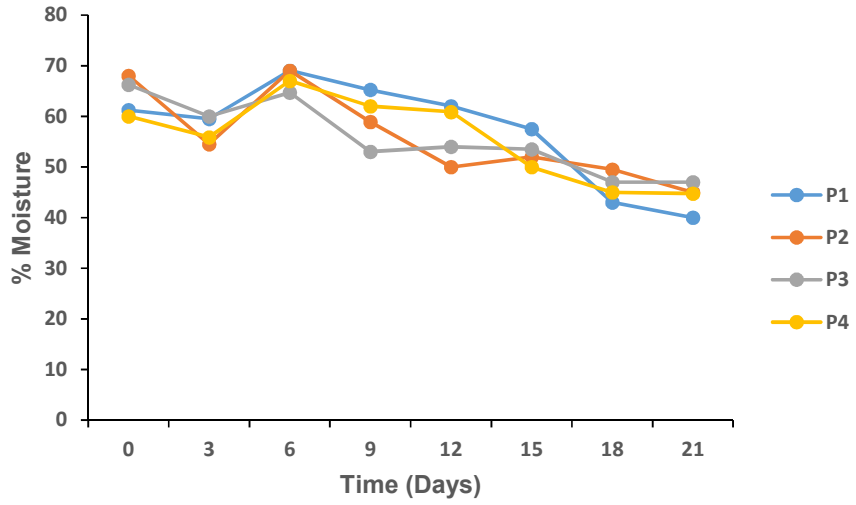

Fig. 7. Moisture (%) of compost produced using effective microorganisms (EM)
P_1: Organic waste + EM A, P_2: Organic waste + EM B, P_3: Organic waste + Commercial EM (EM C), P_4: Organic waste (uninoculated)

Table 2. Moisture content of compost (%)

Time (days)	P_1	P_2	P_3	P_4
0	61.25±1.25^a	68.00±0.00^b	66.25±1.25^b	60.00±0.00^a
3	59.50±0.50^c	54.50±0.50^a	60.00±0.00^c	56.35±0.35^b
6	69.00±1.00^c	69.00±0.00^c	64.75±0.25^a	67.00±0.00^b
9	65.20±0.25^d	58.90±0.10^b	53.00±0.00^a	62.00±0.00^c
12	62.00±0.00^d	50.00±0.00^a	54.00±0.00^b	60.84±1.15^c
15	58.50±0.50^d	52.00±0.00^b	53.50±0.50^c	50.00±0.00^a
18	43.00±0.00^a	50.50±0.50^d	47.00±0.00^c	45.50±0.50^b
21	40.00±0.00^a	45.00±0.00^b	47.50±0.50^c	44.75±0.25^b

^{a,b,c,d,e}: means denoted by different superscripts along the same row are significantly ($p<0.05$) different. P_1: Organic waste + EM A, P_2: Organic waste + EM B, P_3: Organic waste + Commercial EM (EM C), P_4: Organic waste (uninoculated)

wastes inoculated with effective microorganisms, both locally produced (EM-A; EM-B) and commercial (EM-C), resulted in a more uniform textured end-product than the uninoculated treatment. There was no noticeable foul odour in all the treatments at the end of composting.

Table 3 shows the chemical properties of the organic fertilizer, OF_1(Organic fertilizers with EM-A), OF_2 (Organic fertilizers with EM-B), OF_3 (Organic fertilizers with commercial EM, EM-C)and OF_4(Organic fertilizers without EM)as well as that of the fresh organic solid wastes (Feedstock, OF_0). The pH of the organic fertilizers were alkaline (7.50-8.00) at the end of composting process. This may be as a result of the composition of the feedstock [54]. Jawahar [46] and [48] reported that the accumulation of Ammonia (NH_3) released during the decomposition of Nitrogen-containing organic matter dissolves in the moisture present to form alkaline NH^{4+}hence the alkaline pH.

The organic carbon in OF_1 was 15.44%, while OF_2 and OF_3 had 13% and 14% of organic carbon respectively. OF_4 had 17.22% organic carbon. These results demonstrated the enhancement of the rate of biodegradation using EM since a reduction in such parameters as organic matter is an indication of the rate of biodegradation [55]. There were significant differences in the percentage organic carbon of the different treatments at 5% probability level ($P< 0.05$).These results agree with the findings of [56] who reported the percentage of organic carbon in organic fertilizer to range from 10% to 23%. The report of 60) which recorded 16.76% - 18.40% organic carbon agrees with the value in OF_4 of this study. However, [3] and [51] reported 30% - 70% organic carbon in finished compost.

These later values are higher than the values obtained in the present study. Patidar et al. [47] reported that, during composting, part of organic carbon is released as CO_2; part incorporated into microbial cells while part is humified. This could be the reason for lower organic carbon level in the organic fertilizers produced than in the original feedstock.

Total nitrogen values (Table 3) for OF_1 (2.48 g/kg) was comparable with that of OF_3 (2.55 g/kg) and both were higher than that of the feedstock which was 2.00 g/kg as well as those of $OF_2$and OF_4 which were 1.06 g/kg and 1.14 g/kg respectively. The nutrient content of the original waste materials varied with those of the finished compost. The nitrogen level which was at 2.0 g/kg in the organic waste materials showed some changes in the various finished composts. This may be because as decomposition progressed, the Nitrogen which was in organic form got converted to ammonia N and this may volatilize and be lost to the atmosphere or converted to ammonium [56]. The results of this study are in agreement with the findings of [3,54,53] who reported total nitrogen values of 1.0 - 2.0 g/kg,1.35 g/kg and 1.2-2.4 g/kg respectively. The value of total nitrogen as recorded in this study is acceptable since the MSW Compost Quality Standard is recommended for any value greater than one gramme per kilogramme (> 1 g/kg).

The value for available phosphorus in this study ranged from 0.9 to 2.48%. OF_1 had the highest phosphorus value of 2.48 mg/kg, while the other treatments had values higher than the feedstock (0.90%). Phosphorus, which is in organic form and has no volatile-loss pathway, may have had negligible loss, hence recorded higher in all finished organic fertilizers than in the original organic waste materials. This agrees with the report of [53] who recorded 2% - 3.9% while [54] and [3] reported relatively low levels of phosphorus (0.56 and 0.6 - 0.9% respectively). However, according to [50], Councils for Agriculture's recommended level of phosphate in organic fertilizers is 0.3%.

The potassium (K^+) values for OF_2 and OF_3 (2.11 and 2.51 cmol/kg respectively) were significantly ($P<0.05$) higher than that of the feedstock (1.00 cmol/kg) while the values for OF_1 and OF_4 were not significantly ($P<0.05$) higher than the feedstock. Potassium has no volatile-loss pathways and so, composting tends to concentrate it in finished compost. There were no significant differences ($P<0.05$) between the feedstock and the end-product (organic fertilizers) except in OF_2 (Organic fertilizers with EM B), which had a value of 1.76 cmolkg as compared to the original feedstock, with a value of 0.81 cmol/kg. OF_1 (Organic fertilizers with EM A), OF3 (Organic fertilizers with commercial EM, EM-C) and OF_4 (Organic fertilizers without EM), had values of 0.81 cmolkg, 0.91 cmol/kg and 0.82 cmol/kg respectively. The magnesium content of feedstock (0.29 cmol/kg) was significantly different from that of OF_2, OF_3 and OF_4 which were 0.41 cmol/kg, 0.36 cmol/kg and 0.30 cmol/kg respectively. The sodium content of feedstock was 0.3 cmolkg and was lower than that of the organic fertilizers produced. The highest value for sodium was recorded in OF_2 and was 5.21 cmol/kg. The lowest value of 0.45 cmol/kg was recorded in OF_1. Watson, [54] reported K concentration of 0.2 cmol/kg - 0.5 cmol/kg in his study, while he also reported that the Councils for Agriculture approved 0.3% as acceptable value of potassium in organic fertilizers.

Table 3. Chemical properties of organic fertilizers produced using effective microorganisms

Properties	OF_0	OF_1	OF_2	OF_3	OF_4
pH	6.40±0.00^a	8.00±0.00^c	7.70±0.00^b	7.80±0.00^b	7.50±0.00^b
Organic carbon (%)	28.25±0.05^d	13.18±0.00^a	15.44±0.01^b	13.56±0.01^a	17.22±0.00^c
Total Nitrogen (g/kg)	2.00±0.00^b	2.48±0.01^c	2.55±0.00^c	1.06±0.00^a	1.14±0.00^a
Available phosphorus (mg/kg)	0.90±0.00^a	2.48±0.00^b	2.41±0.15^b	1.06±0.01^{ab}	1.00±0.00^{ab}
Potassium (cmol/kg)	1.00±0.00^a	2.51±0.01^b	2.11±0.01^b	1.05±0.00^a	1.16±0.00^a
Calcium (cmol/kg)	0.81±0.01^a	0.81±0.01^a	1.76±0.01^c	0.91±0.01^{ab}	0.82±0.01^a
Magnesium (cmol/kg)	0.29±0.00^a	0.36±0.01^b	0.41±0.01^c	0.26±0.01^a	0.30±0.00^b
Sodium (cmol/kg)	0.34±0.01^a	1.67±0.01^d	5.21±0.01^e	0.45±0.01^b	0.86±0.01^c
Organic matter (%)	25.58±0.00^b	29.81±0.01^c	23.45±0.01^b	22.75±0.00^a	21.53±1.50^a

^{a,b,c,d,e}: means denoted by different superscripts along the same row are significantly ($p<0.05$) different.

OF_0: Organic waste materials (Feedstock), OF_1: Organic fertilizers with EM-A, OF_2: Organic fertilizers with EM-B, OF_3: Organic fertilizers with commercial EM, OF_4: Organic fertilizers without EM

The results of organic matter content of the organic fertilizer produced in the present study showed low values (21.53 - 29.81%). The highest organic matter content (29.81%) was recorded in OF_1. The values for other treatments OF_2, OF_3 and OF_4 were 22.75%, 23.45% and 21.53% respectively, and these were lower than that of the feedstock, which had 25.58%. Comparing the results with the standards of compost, acceptable organic matter content is any value greater than 20%. Organic matter content reported by [1] ranged from 25% to 35%.

The chemical composition of MSW (feedstock) in this study agrees with the standard values suitable for composting according to [27]. The investigators reported a pH range of 5.5 - 8.0, moisture content of < 50, organic matter - > 20, Nitrogen > 0.6 and carbon 30% - 40% as standard [27].

4. CONCLUSION

The activities of the microorganisms that constituted the effective microorganisms were responsible for the reduction of pH of the EM solution within 3 days of incubation. During composting of the organic municipal solid wastes, EM-A enhanced the rate of biodegradation of the organic wastes higher than other EM. The higher loss of weight of organic waste materials in composts where EM was applied more than the uninoculated treatment is an indication that the use of EM ensured faster and better waste degradation. OF_1 (Organic fertilizers with EM-A), which had the best fertilizer value based on the N-P-K values is considered most suitable for crop use.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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Peer-review history:
The peer review history for this paper can be accessed here:
http://sciencedomain.org/review-history/21794