

COMPOST MATURITY AND GERMINATION INDEX PROFILE OF MUNICIPAL SOLID WASTE MATERIALS COMPOSTED WITH ORGANIC ADDITIVES**Ofunwa, J.O.¹, Mbachu, I.A.C.² and Umeaku, C.N.²**¹Department of Microbiology, Faculty of Natural and Applied Sciences, Tansian University, P.M.B. 0006 Umunya, Anambra State, Nigeria.²Department of Microbiology, Chukwuemeka Odumegwu Ojukwu University, P.M.B.02 Uli, Anambra State, Nigeria.Correspondence: joypatofunnwa2020@gmail.com; +2348063981789.

Abstract

Composting municipal solid waste is a viable organic waste management strategy, and with the potential to enhance waste management system. However, a conventional composting is rarely applied due to the longer time consumed to reach maturity phase. The present study was undertaken to investigate the compost maturity and germination index profile of composted municipal solid waste materials with organic additives. Effective microorganisms (bacteria and yeast strains) isolated from plant root, soil and fruit samples were employed in the biosynthesis of nanocomposites using standard microbiological and nanotechnological techniques. The nanocomposites and effective microbes were applied in the biocomposting of 5.5 kg shredded and dried final waste mixture of approximately 55 % food waste, 15 % saw dust waste, grass chopping waste 22 % and 8 % paper and control for 56 days. The waste materials and compost end products were analysed for chemical and plant seed growth characteristics like total organic carbon, nitrogen, carbon nitrogen ratio and germination index on *Zea mays* and *Glycine max* seeds using standard procedures. The result revealed that paper waste had the highest carbon nitrogen ratio value of 173:1 while food scrap waste had the lowest carbon nitrogen ratio value of 24:1, respectively. Also, after 56 days of the composting, the magnesium nanocomposite setup had the highest germination index value of 88.00 % while control set up had the lowest germination index value of 57.00 % for *Z. mays* seeds. Furthermore, the magnesium nanocomposite setup had the highest germination index value of 93.00 % while control set up had the lowest germination index value of 62.00 % for *G. max* seeds, respectively. Statistical significant differences ($P < 0.05$) were found in GI parameters among the means of treatment set ups relative to control set up. Thus, all the compost treatment regimen do not have phytotoxicity effect and are potential phytonutrient or phytostimulant to *Zea mays* and *Glycine max* seeds while the control setup had moderate phytotoxicity to both plant seeds, respectively.

Keywords: Compost, Germination index, *Glycine max*, *Zea mays*, Phytotoxicity

INTRODUCTION

Municipal solid waste (MSW) comprises waste from household, offices, markets, construction and demolition debris, dead animals and abandoned vehicles and public places and its management is presently an issue of global concern (Niazi *et al.*, 2016). Very high volumes of municipal solid waste are being generated particularly in urban areas, and are increasing rapidly as a result of population growth and changing consumption pattern resulting from changing economic situations. The challenges of municipal solid waste management are particularly grave in developing and low-income countries, where waste management is primarily the responsibility of local authorities (Guerrero *et al.*, 2013). On average up to 50 % of residents lack collection services in urban areas of low- and middle-income countries (Tibu *et al.*, 2019).

Composting is a biological method whereby consistent introduction of air by mechanical turning rouses oxygen consuming microorganism to diminish natural materials such as manure to a more steady material like humus. It is an appropriate method for reusing natural squanders in an ecologically benevolent way. Due to the sorts, nature, and composition of wastes in developing countries, composting remains the most sparing and proficient treatment technique among other treatment techniques. Further to serving as a waste management strategy, composting organic waste will serve as a source of employment and revenue generation for the youth, farmers, and others who take part in the recovery, processing, and utilization chain. It will also provide an environmentally sound alternative to inorganic fertilizer in replenishing farmlands, especially in cases where source segregated organic waste are used to reduce the chances of toxic household chemicals getting infused into the compost produced, thereby introducing toxins into the soil and

plants (Tibu *et al.*, 2019). However, the application of immature compost may restrict plant growth through competition for oxygen in the rhizosphere and release of toxic substances.

Therefore, evaluation of compost maturity is extremely important. Physical, chemical, and biological methods can be used to characterize the change in compost maturity (Komilis, 2015, Chen *et al.*, 2018). Among these approaches, the seed germination index (GI) has been widely used as a biological indicator to evaluate the phytotoxicity and maturity of compost (Luo *et al.*, 2018). The GI was first proposed by Zucconi *et al.* (1981) and includes the effect of phytotoxic substances on both the germination rate and radicle elongation of seedlings. The most recent Chinese agricultural industry standard, ‘Technical Specifications for Composting of Livestock and Poultry Manure’ released in 2019, stipulates that the GI of mature compost should be $\geq 70\%$. China’s newly revised standard for organic fertilizer (NY525-2021) includes a requirement for $GI \geq 70\%$ (Yang *et al.*, 2021). The GI is affected by many factors, including the species, seed culture medium, and cultivation environment. The relevant properties of compost extract mainly comprise electrical conductivity (EC), pH, E_4/E_6 ratio, ammonium-nitrogen ($\text{NH}_4^+\text{-N}$) content, and heavy metal concentrations, all of which may affect seed germination. In addition, the relevant qualities of seeds include growth rate, sensitivity, and tolerance, which may lead to differences in seed sensitivity to toxic substances. The determination of GI is regulated by the Chinese standard for organic fertilizer (NY525-2021), in which the incubation period is prescribed as 48 h at 25 °C. Therefore, the procedure requires seeds that germinate rapidly, especially in deionized water, and the germination percentage and root length in the control experiment are crucial to the accuracy of GI determination (Yang *et al.*, 2021).

To date, no standard has been proposed for seeds used to determine the GI and the selection of seeds in published research varies widely. Chen *et al.* (2020) used corn seeds to explore the phytotoxicity of antibiotics in the co-composting of food waste and sewage sludge. Wu *et al.* (2018) selected corn seeds to study the effect of different types of compost on the seed germination percentage, seedling biomass, and root vitality. Those studies are limited in that there is paucity of information on phytotoxicity or germination index profile of effective microorganisms and nano-agents of biological origin either as single or combined additives during composting of municipal solid waste, hence justify the current study. Thus, the objective of this paper is to investigate the compost maturity and germination index profile of municipal solid waste materials composted with organic additives in Ihiala local Government Area, Anambra State, Nigeria.

MATERIALS AND METHODS

Material and Sample Collection

Pure magnesium nitrate (MgNO_3) reagents and other chemicals of analytical grade that will be used in this study will be obtained from Loba Chemie, Mumbai India. Rhizospheric soil samples and roots of soy beans were aseptically collected with sterile hand trowel and knife from the school garden within premises of Chukwuemeka Odumegwu Ojukwu, Uli Campus Ihiala Anambra State while ripe queen pineapple (*Ananas comosus*) specimens were bought from Nkwo Ogbe Market Ihiala Anambra State. All the samples were placed into sterile polyethylene bags and transported on ice to the Microbiology Laboratory of Chukwuemeka Odumegwu Ojukwu University Uli Campus, Nigeria for further analysis.

Enrichment and Isolation of Effective Microbial Species

The methods of Ogbo and Okonkwo (2012), Jahangir *et al.* (2019) and Umeh *et al.* (2019) were adopted in the isolation of rhizospheric bacterial (RB) species, phosphate solubilizing bacteria (PSB) and yeast using nitrogen free biotin medium (NFb), Pikovaskaya (PVK) agar medium and Yeast Extract Dextrose Peptone broth, respectively. After incubation, discrete colonies were

selected and purified cultures were preserved in 20 % glycerol contained in Bjou bottle and stored at - 70 °C.

Magnesium Nanoparticle and Nanocomposite Biosynthesis

The modified methods of Kazemi *et al.* (2020), Hassan *et al.* (2021) and Saied *et al.* (2021) were adopted in the biosynthesis of magnesium nanoparticles and nanocomposites using mixture of bacterial and fungal filtrates under magnetic stirrer for 120 min, 70 °C and 80 rpm.

Composting

Seed culture

The modified methods of Limaye *et al.* (2017) was adopted for the composting method. Four strains selected for the formation of consortium were designated as: Rhizospheric bacteria (RB), Phosphate solubilizing bacteria (PSB) and Yeast strain (YS). These strains were used as the seed culture for composting.

Inoculum build up and formulating the consortium of selected strain

The three selected strains were grown at room temperature for 36 h on nutrient broth medium until maximal exponential growth was reached. For each strain, bacterial and yeast suspensions were prepared in saline with 0.01 % Tween – 80. Pooled bacterial and yeast suspensions were prepared by adding 100 mL suspension of each strains.

Substrate collection and preparation

The food waste consisting of leftover food, fruit and vegetable were collected from the eatery centres, restaurants, local markets and vendors in Uli town. Saw dusts were collected from timber sheds and local carpentry workshops at Ihiala town. The grass straws were collected within Chukwuemeka Odumegwu Ojukwu University, dried and chopped into pieces. The paper waste consisting mostly of unused office paper and tissue paper were gathered from within the

Chukwuemeka Odumegwu Ojukwu University Uli campus. All safety measures when handling these wastes such as wearing rubber gloves and face masks are observed. All the non - compostable materials contained in the waste were sorted out and not included in the compost preparation. The food waste samples were then rinsed with the tap water for removing the oil and impurities. The organic wastes were air - dried for a couple of days to remove the excessive moisture. The sorted organic materials were crushed to fine particles and then transferred to a rectangular composter as a raw material for composting (Ali *et al.* 2013; Limaye *et al.* 2017; Njoku *et al.*, 2019a, b; Saleh *et al.* 2020). All sample collection centers were located in Ihiala Local Government Area, Anambra State, Nigeria, respectively.

Composting unit

The passive aeration composting experiment was carried out in 13 L laboratory made plastic bin composter with dimensions 43 cm x 32 cm x 25 cm (length x width x height) and aeration holes (0.6 cm diameter) at the sides of the bin. Each experiment contained 5.5 kg shredded and dried final waste mixture of approximately 55 % food waste, 15 % saw dust waste, grass chopping waste 22 % and 8 % paper with or without inoculation as described by Aslanzadeh *et al.* (2020). The first set up was added the bacterial and yeast suspension labelled effective microorganisms, the second set up was magnesium nanoparticles labelled Mg nanocomposite, the third set up was added a combination of both microbial suspension and magnesium nanocomposite labeled consortium while the fourth set up labelled uninoculated control was maintained with saline of 0.01 % Tween -80, respectively (Zhao *et al.* 2017; Saleh *et al.* 2020).

Maintaining the moisture and aeration level

Composting units were kept in the laboratory shade. Composting was allowed to take place for 8 weeks. Moisture level was maintained by addition of 20 mL sterile water to the pile every day. All

the samples were aerated by turning the pile using sterile plastic rod every day in the first two weeks and after that only once a week for the rest of the experimental period. The experiment was carried out in triplicates (Limaye *et al.* 2017; Aslanzadeh *et al.* 2020).

Analysis of the composted material

During the 56 days composting period, the following parameters for the experiments were measured as follow:

Carbon Nitrogen ratio analysis

Total organic carbon determination

The total organic carbon (TOC) of the individual waste materials were determined by partial oxidation method (20) through titration against 1N $(\text{NH}_4)_2\text{Fe}(\text{SO}_4)_2 \cdot 6\text{H}_2\text{O}$ using diphenylamine indicator as described by Ataikiru *et al.* (2019). In this test, 10.0 mL of 1N potassium dichromate was added to 1 g of grounded sample in a 250 mL Erlenmeyer flask, and the mixture was gently shaken to disperse soil. Thereafter, 20 mL concentrated tetraoxosulphate (iv) acid was added, swirled and allowed to stand for 30 min. A 100 mL distilled water was added, followed by the addition of 10 mL O - phosphoric acid. Three to four drops of the indicator was added and titrated with 0.5 N ferrous ammonium sulphate till colour changed from green to blue and finally to red (end point).

Total nitrogen determination

The Kjeldahl spectrophotometric method was adopted according to the modified methods of Okalebo *et al.* (2002) and FAO (2008). One gram of grounded samples was weighed and placed in a Kjeldahl flask. Kjeldahl catalyst was added with composition: 0.358 g of sodium sulphate, 0.358 g of potassium sulphate, 0.1415 g of titanium oxide, 0.1415 g of copper sulphate with 10 mL of H_2SO_4 , 2 mL silicon oil and 3 - 5 glass beads to reduce frothing. The mixture was heated

gently until frothing ceases. It was later boiled briskly until the solution is clear and then the digestion continued for at least 30 min. Dilute all the digested sample, standards and the blanks to 1 + 9 (v/v) with distilled water to match the standards. With the aid of a pipette, take 0.2 mL of the digested sample, standard and the blank into clearly labelled test tube and add 5.0 mL of the reagents N1 and N2 and vortexed simultaneously. Allow standing for 2 h to observe blue colouration and measure the absorbency at 650 nm. Plot a calibration curve and read off the concentration of N in the solution.

The nitrogen concentration in the sample material expressed in %N is calculated as follows:

$$N (\%) = \frac{(a - b) \times v \times 100}{1,000 \times w \times al \times 1,000} \quad \text{----- Equation (9)}$$

where a = concentration of N in the solution, b = concentration of N in the blank, v = total volume at the end of analysis procedure, w = weight of the dried sample and al = aliquot of the solution taken.

Compost Phytotoxicity Test

The experiment was carried out using water extracts of the four different composted materials prepared according to the procedure described by EBPI (2016), Uba *et al.* (2021) and Sobieraj *et al.* (2022) for seed germination and early growth microbiotest with higher plants –Phytotoxkit. First, a test of water holding capacity of the reference soil was performed by filling a 100 cm³ cylinder with 90 cm³ of soil sifted through a sieve with a mesh size of 2 mm and 50 cm³ of distilled water. It was mixed until the soil was completely saturated with water. After reaching the equilibrium state, the supernatant was poured into a 50 mL measuring cylinder and used to determine the water holding capacity of the reference soil. Then for each compost treatment, the 90 cm³ of reference soil was placed on a transparent PVC test plate composed of a bottom

compartment and a flat cover. Subsequently, 30 cm³ of water extract was measured and introduced into the reference soil containing a paper filter on its surface. Thereafter, 10 seeds each of *Zea mays* and *Glycine max* were placed in one row at the same intervals from each other. The PVC plates were covered and appropriately labelled with respective samples in three sets. The test plates (samples and controls) inoculated with the test plant seeds were carefully placed in the incubator (Kottermann D3165, West Germany) and incubated at 25 ± 2 °C for 3 days in darkness. After the 3 days of incubation, the number of germinated seeds and the length of each roots were counted and measured and the mean triplicate values were used to determine the germination index defined as:

$GI = (Gs/Gc) \times (Ls/Lc) \div 100$ where Gs and Gc are mean germinated seeds in the sample and control; and Ls and Lc are mean root elongation in the sample and control respectively.

Statistical Analysis

Data were analyzed using GraphPad Prism Version 8.0.2. Descriptive statistics were performed to summarize the data in the form of mean. The Two - Factor Analysis of Variance (ANOVA) followed by Dunnett's multiple comparison test was adopted in comparing the germination indices of the consortium, effective microorganisms and magnesium oxide nanocomposite with respect to their controls at 95 % confidence interval and P values below 0.05 were considered significant.

RESULTS AND DISCUSSION

A nutritional balance in form of an optimum C/N ratio is essential to formulate an efficient compost mix. As composting proceeds with time, variations in C/N projected the rate of organic degradation as governed by the extent of carbon transformed to CO₂ (Ralstogi *et al.*, 2020). Researchers have

suggested various ideal or recommended range of C/N ratios ranging from 12 to 25 but the optimal value is often dependent on the initial feedstock (Pushpa *et al.*, 2016; Mamo *et al.*, 2021). The result in Table 1 represents the chemical parameters of the individual wastes materials used in composting. From the results, paper waste had the highest carbon nitrogen ratio value of 173:1 while food scrap waste had the lowest carbon nitrogen ratio value of 24:1, respectively.

Table 1: Chemical parameters of the wastes used in composting

Parameter	Food scrap waste	Saw dust waste	Grass chopping waste	Paper waste
Total carbon (%)	33.00	170.00	154.00	249.00
Total nitrogen (%)	1.40	2.50	3.12	1.44
C:N	24:1	68:1	49:1	173:1

The germination test has been used to evaluate the compost maturity and phytotoxicity of organic and inorganic wastes and assess its suitability for use as soil amendment or growing media (Tibu *et al.*, 2019; Mamo *et al.*, 2021). The seed germination index (GI) test helps to evaluate the efficiency of the composting process for plant growth (Kazemi *et al.*, 2017). The result in Figure 1 represents the changes in germination index of *Zea mays* seeds exposed to composted materials during 56 days composting period. On the day 1 of the composting, the consortium setup had the

highest germination index value of 16.00 % while effective micro-organisms setup had the lowest germination index value of 11.00 %. After 56 days of the composting, the magnesium nanocomposite setup had the highest germination index value of 88.00 % while control set up had the lowest germination index value of 57.00 % respectively. The result in Figure 2 represents the changes in germination index of *Glycine max* seeds exposed to composted materials during 56 days composting period. On the day 1 of the composting, the magnesium nanocomposite setup had the highest germination index value of 16.00 % while control setup had the lowest germination index value of 9.00 %. After 56 days of the composting, the magnesium nanocomposite setup had the highest germination index value of 93.00 % while control set up had the lowest germination index value of 62.00 %, respectively. The results in Figures 1 and 2 showed that there were lower GI values at the beginning (Day 1) to day 28 in all the composting units for both *Zea mays* (Figure 1) and *Glycine max* (Figure 2) tested seeds. The possible reason for this observation could be due to the presence of phytotoxins such as phenolics, volatile or water organic acids, alcohols, ethylene, ammonia, salts, heavy metals which are inherent or produced in the composting feedstocks or system. As the composting period progressed, there were attainment of significant GI values such that over 50 % and 80 % were obtained in *Zea mays* seed test for control and treatment setups and over 60 % and 90 % in *Glycine max* seed test for control and treatment setups at the end of the 56 composting experiments. The possible reason for these very higher GI values could be said to be the disappearance of phytotoxins as results of their bioconversion into less toxic forms by mesophilic microorganisms inherent in the composted materials. A germination index values below 50 % indicate high phytotoxicity; value between 50 and 80 % indicate moderate phytotoxicity; and values above 80 % indicate the absence of phytotoxicity. When the index exceeds 100%, the compost can be considered as a phytonutrient or phyto stimulant (Mamo *et al.*,

2021). This implied that all the compost treatment regimen do not have phytotoxicity effect and are potential phytonutrient or phytostimulant to *Zea mays* and *Glycine max* seeds while the control setup had moderate phytotoxicity to both plant seeds. Statistically, significant changes ($P < 0.05$) were detected in the *Zea mays* seed GI test and *Glycine max* seed GI test among the means of treatment set ups relative to control set up using two factor Analysis of Variance (ANOVA). Maturity index classification for seed germination as proposed by Briton (2000) rated GI values $> 90\%$ very mature; GI values $80 - 90\%$ mature and GI values $< 80\%$ immature, respectively. The GI values obtained in this study rated the treated composting units having very mature to mature composted materials after 56 days of composting while the control setup was rated immature by the classification above. These observations was confirmed by the previous published research works of Selim *et al.* (2012), Kazemi *et al.* (2017b), Tibu *et al.* (2019), Jagadagbi *et al.* (2019), Anukam *et al.* (2020) and Mamo *et al.* (2021) on MSW, market waste, fish waste, coffee pulp waste and plant waste composts, respectively and therefore support the findings of the present study.

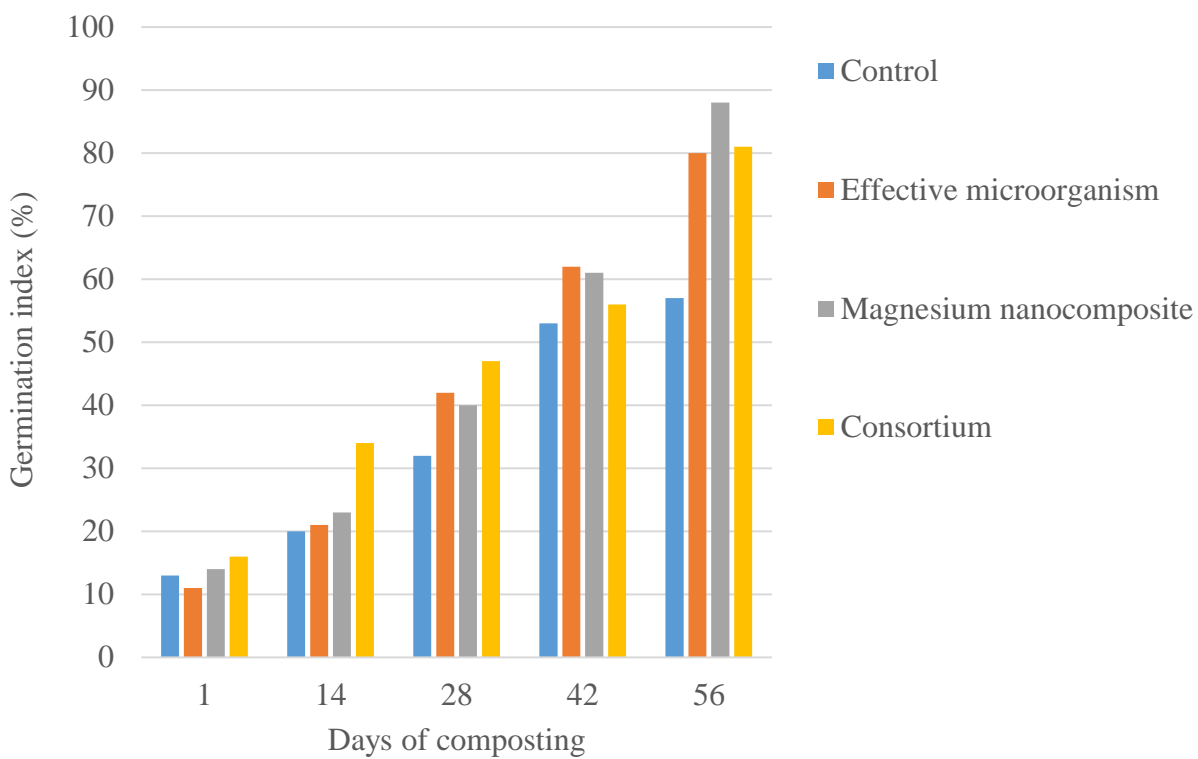


Figure 1: Changes in germination index of *Zea mays* seeds exposed to composted materials during 56 days composting period

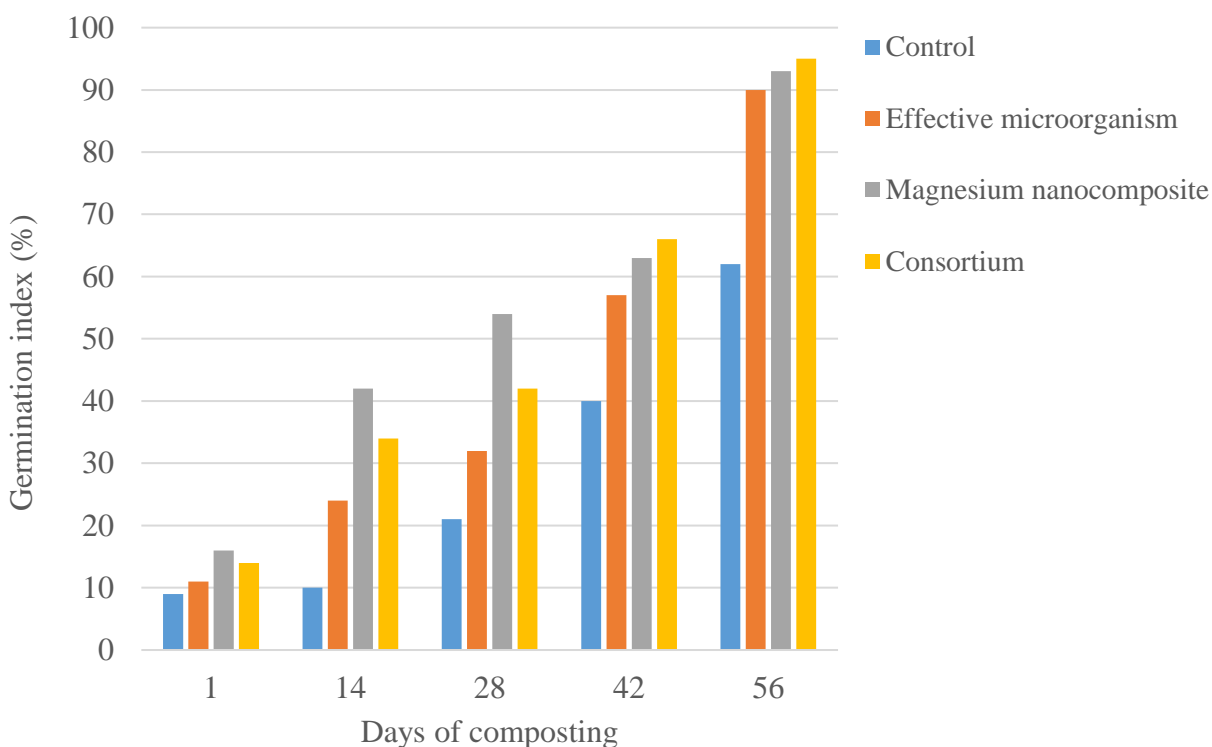


Figure 2: Changes in germination index of *Glycine max* seeds exposed to composted materials during 56 days composting period

CONCLUSION

The whole study revealed that the municipal solid wastes mixed effective microorganisms and magnesium nanocomposite either in single or combined form are potential composting materials.. Statistical significant differences ($P < 0.05$) were found in GI parameters among the means of treatment set ups relative to control set up. Hence, proper means of disposing, converting and

recycling of these waste materials as well as formulation of nanobiofertilizers should be encouraged and implemented by the environmental stakeholders, general public and government.

ACKNOWLEDGMENTS

We wish to appreciate Chikelue Chisom Faith and Academic Technologists of Microbiology Department of Chukwuemeka Odumegwu Ojukwu University, Uli Campus for their Technical Assistances towards the completion of this project work

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