DETERMINATION OF THE EFFECT OF MIXED MICROBIAL CULTURES ON THE ANTINUTRIENTS COMPOSITION OF COMPOST (DOMESTIC FOOD WASTE)

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Abstract

This paper assessed the degradative activity of mixed microbial cultures on the anti-nutrient composition of domestic food wastes (DFW) used for composting. The domestic food wastes were inoculated with the mixture of the microorganisms, left to decompose for 42 days at ambient temperature (28°C) during which samples were taken at 7-day intervals for the determination of the anti-nutrient contents of the DFW using standard chemical methods. Different species of fungi namely Aspergillus flavus, Aspergillus fumigatus, Varicosporium elodeae, Trichodermaroseum, Penicillium italicum and Rhizopus nigricans were found to inhabit the domestic food wastes. The test bacteria used were confirmed to be Lactobacillus delbrueckii, Geobacillus stearothermophilus, Bacillus megaterium, Lactobacillus jensenii, Bacillus sphaericus, Macromonnas mobilis, Azotobacter, Listeria monocytogenes and Kurthia species. These organisms caused a significant reduction in the anti-nutritional contents of the composts all through the composting period.

Keywords: Compost, Composting, Domestic food wastes, Anti-nutrient, Degradation

1. Introduction

Composting is a process that results from biodegradation. Composting by definition is the biodegradation of fibrous materials to create usable forms of fertilizer (Gross, 2002). It is a microbial decomposition or rotting process where organic substances in organic solid wastes are subject to biological break down into simpler forms of matter in a moist, warm and aerated environment to produce compost which is a mixture of decaying organic matter (Eslava and Garcia, 2001).

Decomposition is an integral part of the natural life cycle (Heider and Rabus, 2008).In composting, the biological decomposition of the organic constituents of wastes is subjected to controlled conditions (Gen et al., 2006). The application of the controlled condition distinguishes composting from putrefaction or other decompositions that takes place in an open dump, sanitary landfill, and manure heap or in an open field (Fu and Chen, 1990). By the intentional act of composting, humans participate in what has been called "Nature's law of return" due to a vital link established for the return of organic matter to soil system (Chen-Chin et al., 2009). Composting biodegrades organic wastes transforming its products into a nutrient rich component that is capable of improving depleted or disturbed soil environment (Cornell Waste Management Institute, 2000). Composting has been a way to recycle waste and a critical means in reducing the volume of garbage needlessly sent to landfills and also provides a means of supplying necessary nutrients to plants (Cornell Waste Management Institute, 2004). Food wastes are organic waste materials that are either raw or cooked that are discarded intentionally or unintentionally (Anonymous, 2005b). The composting process is currently viewed primarily as a waste management method to stabilize organic waste, such as manure, yard trimmings, municipal bio solids, and organic urban wastes. The stabilized end-product (compost) is widely used as a soil amendment to improve soil structure, provide plant nutrients, and facilitate the revegetation of disturbed or eroded soil (Apun et al., 2000).

Domestic food waste (DFW) ranging from inedible food waste from in-home preparation activities (for example peelings, leaves, trimmings, bones, tea bags and other packaging materials) to edible waste (such as food left uneaten and out of date food) are generated at a very high rate globally from homes and several institutions. In most cities of Nigeria, DFW constitute a very high percent of dumps at dump sites(Bergqvist*et al.*, 2005).

Inorganic fertilizers imported into Africa cost two to six times as much as those in Europe, North America or Asia (Tiquiaet al., 2002), making it inaccessible to smallholder farmers. The rising cost of inorganic fertilizers coupled with their inability to condition the soil has directed attention to organic manures in recent times. Compost is biologically active. When this product is ploughed into the soil, it supplies a range of microorganisms increasing soil's microbial diversity, populations and activity (Arnedo and Parrado, 2002). The combined interaction of several microorganisms in the decomposition process results in compost that contains significant quantities of organic matter (BBC Laboratories, 2004). This work, assessed the effect of mixed cultures of microorganisms isolated from domestic food wastes on the anti-nutrients composition of the composts.

2. Materials and methods

The domestic food waste (DFW) used were: vegetables ('Ugu': Telfairiaoccidentalis) 'Tete': Amaranthus species), pulp and peels of banana and oranges, boiled rice grain (Capricorn Brand, Thailand), green grass (Centrosema pubescence) and chicken droppings. The test bacteria Lactobacillus delbrueckii, Geobacillus stearothermophilus, Bacillus megaterium, Lactobacillus jensenii, Bacillus sphaericus, Macromonnas mobilis, Azotobacter, Listeria monocytogenes and *Kurthia* species were used in this study.

Sample collection

Samples of domestic food wastes (DFW) were collected at different depths (2 cm, 5 cm and 10 cm) of the dump wastes in Akure main dumpsite situated at Oda road Akure, Ondo State. Individual DFW listed above were collected from Akure main market into separate sterile plastic bowls.

Preparation and sterilization of media:

The media used in this experiment for the isolation of microorganisms are Nutrient Agar(NA) and Saboraud dextrose Agar (SDA), Nutrient broth (NB), Sabouraud dextrose broth (SDB) and Agar-agar. The media were prepared following the standard laboratory methods as described by Cheesebrough (2003).

Isolation of Fungal and Determination of Microbial loads

One gram of each domestic food waste (DFW) sample collected was weighed into 9ml of sterile water to make a mixing stock. The mixture was serially diluted by taking 1ml of stock mixture into appropriately labelled test tube to make 10^{-1} of the mixture. The serial dilution was continued until 10^{-10} was obtained. An aliquot (0.1 ml) of the respective dilutions was pour plated using Nutrient agar (NA) and Sabouraud dextrose agar (SDA). The inoculum in NA and SDA were incubated at 37° C $\pm 2^{\circ}$ C for bacterial and at 28° C for 72 to 120 hours for fungi. Cultural features of the fungi were also observed (Onions *et al.*, 1981).

Preparation of Domestic Food Wastes Sample

Domestic food wastes (DFW) was reduced into smaller pieces using a kitchen blender (Master Chef Model AA4) and shredded using sterile table knife. The wastes were blended separately and pooled together. They were mixed thoroughly together and weighed in equal proportions of (1200 grams) into labeled plastic bowls. The wastes were then sterilized in an autoclave at 121°C for 15 minutes and allowed to cool. The control treatments were thus named: CD: Unsterilized domestic food wastes from Akure dump site, CC- Constituted Domestic food wastes: Domestic food waste (DFW) collected from the "Oba" market in Akure, CSWM: Sterilized uninoculated (made without microorganisms) domestic food wastes from the "Oba" market food wastes from the "Oba" market and CSM: Sterilized inoculated (containing all microorganisms) domestic food wastes from Akure main market.

Inoculation of the prepared domestic food wastes

The authenticated bacteria were inoculated separately into sterile (10 ml) nutrient broth incubated at 37°C for 24 hours. Each fungus was inoculated into Saboraud dextrose broth and incubated at 28°C for 48 hours. The optical density of each grown cell and mycelium was determined at 670nm. The grown cells were combined into twos based on mixing bacterium with fungus, bacterium with bacterium, fungus with fungus, and inoculated aseptically into each separate portion of the sterilized DFW. Thus, the total number of combinations was seven (*Lactobacillus delbrueckii* + *Geobacillus stearothermophilus, Bacillus megaterium* + *Lactobacillus jensenii, Bacillus sphaericus* + *Macromonnas mobilis, Azotobacter* + *Penicillium italicum,Listeria monocytogenes* + *Aspergillus niger, Kurthia* species+ *Aspergillus niger* and *Varicosporium elodeae* +*Rhizopus nigricans*). They were left to decompose for 42 days at a room temperature of 28°C during which each sample was watered with sterile water (5 ml) and turned with a sterile spoon for good aeration. Samples were taken aseptically at 7 days intervals for microbial and physiochemical analyses. At the end of the 42 days, the decomposed domestic food wastes were referred to as composts.

Curing of the Compost

The compost was left to cure for two months at 28°C. During the curing process, new organic material was not added to the compost pile. Weekly the compost pile was aerated by turning it using a sterile spoon and moistened weekly with sterile water (5 ml) to speed the curing process. At 8 weeks the compost texture was damp-sponge-like. The compost was left to shrink in height after which the compost pile was observed at week 10 when it still contained large particles. The compost pile was then left for another one month, after which there was a great reduction in particle size (Tserovska*et al.*, 2002).

Determination of Antinutrient Content

Fifty grams of the composting wastes and composts were taken aseptically for physicochemical analysis at seven day intervals. Antinutrients contents of each sample were determined using the methods described below:

Determination of saponin content

Determination of saponin was carried out according to A. O. A. C. (2000) .One gram metric method employing the use of soxhlet extractor and two different organic solvents wasused. The first solvent extracted lipids and interfering pigments while the second solvent extracted saponins proper. Five grams of the ground sample was weighed into a thimble and transferred into the soxhlet extractorchamber fitted with a condenser and flask. Somequantity (5 ml) of petroleum spirit (with boiling point $40 - 60^{\circ}$ C) enough to cause a reflux was put into the flask. Extraction continued for 3 hr, which extracted the lipids and interfering pigments. The defatted material in the thimble was then used for the second extraction of saponin. A fresh reweighed flask was fitted to the soxhlet apparatus (bearing the thimble containing the defatted sample) and methanol was put in the flask. The quantity of methanol (5 ml) was should be

enough to reflux andflush for 3 hours. The saponin was exhaustively extracted by heating the flask on a heating mantle. After the extraction the thimble and its content were removed and the methanol was recovered leaving the saponin and littlequantity of methanol in the flask. It was then taken to an oven and kept at slanting position at a temperature of 70°C to evaporate the residual methanol. The flask and its content were weighed and the difference between the weight of the flask plus saponin and the weight of the flask alone was the mass of saponin extracted.

% Saponin = $\frac{\text{Mass of saponin in g x } 100}{\text{Mass of sample}}$

Tannin content: The method described by Markkar*et al.* (1993) was adopted for the determination of tannin content. A 400 mg of the samples were placed into one conical Absorbance was taken at using 256nm spectrophotometer and concentration was estimated from the tannic acid standard curve previously plotted using various concentrations of tannin with their corresponding absorbance readings at 256nm.

Evaluation of total cyanide content: Two grams of each sample was weighed into a flask100 ml of distilled water was added to it and allowed tohydrolyze for 1 hr. A 10ml of 2.5% (w/v) NaOH was carefully poured into the sample holder. Thesoxhlet apparatus was set up and the sample was distilled into thesample holder containing the Sodium Hydroxide (NaOH) until about70 ml was collected. It was then carefully transferred to a100 ml volumetric flask and the sample holder was rinsedwith distilled water successively and also poured into thevolumetric flask. The volume in the volumetric flask was made up to the 100 ml mark. Twenty fivemillilitrer (25 ml) of the distillate was pipetted into a conical flask, 2 ml of 6M NH₄OH was added and0.5ml of 10% KI solution was titrated with 0.02M AgN0₃to a first turbid colour(A. O. A. C., 2000).Amount of total cyanide was calculated using the formula written below: 1ml of 0.02 M AgN03 = 1.08mg cyanide

Quantification of phytate: Concentration of phytic acid of each sample was determined using the procedure described by Markkar*et al.* (1993). A 2.0 g of the sample was weighed into 250 ml conical flask. A 100 ml of 2% concentrated HCL acid was used to soak the sample in the conical flask for 3 h and then filtered through a double layer of hardened filter papers. A 50 ml of the

filtrate was placed in 250 ml beaker and 100 ml of distilled water was added to give proper acidity. A 10 ml of 0.3% (w/v) ammonium thiocyanate solution was added to the solution as indicator. The solution was titrated with standard iron chloride solution, which contained 0.00195g iron per ml. The end point color was slight brownish yellow which persisted for 5 min.

Measurement of oxalate content: Oxalate concentration was determined using the method of Oke (1969). A 1.0 g of the samples were placed each in a 250 ml volumetric flask, 190 ml of distilled water and 10 ml of 6 m HCL were added. The mixture was warmed on a water bath at 90°C for 4 h and the digested samples were centrifuged at a speed of 2,000 rpm for 5 min. The supernatant was then diluted to 250 ml. Three (3) 50 ml aliquots of each supernatant were evaporated to 25 m l, and then the brown precipitate was filtered off and washed with distilled water. The combined solution and washings were titrated with concentrated ammonia solution in drops until the critical Salmon pink colour of methyl orange changed to faint yellow. The solution was heated on a water bath to 90°C and the oxalate was precipitated with 10 ml of 5% (w/v) calcium chloride (CaCl₂) solution. The solution was allowed to stand overnight and then centrifuged at 90 revolutions per minute (rpm) for 15 minutes. Each precipitate was washed into a beaker with hot 25% H₂SO₄, diluted to 125 ml with distilled water and was titrated against 0.05 m KMnO₄

3. Results

The saponin in all the DFW were degraded all through 6weeks (Figure 1) while samples T (*Lactobacillus delbrueckii* + *Geobacillus stearothermophilus*) and S (*Listeria monocytogenes* + *Aspergillus niger*) had the highest and lowest comparative mean values of $2.79 \pm 0.01\%$ and $1.52 \pm 0.01\%$ respectively at week 6. The CSM had the lowest value among all the control samples from 0-6weeks with a mean value of $1.42 \pm 0.01\%$ at week 6.

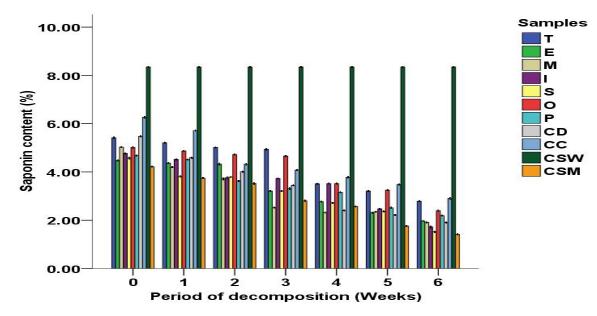
Out of all the decomposing DFW, CD had the lowest oxalate content of $1.74 \pm 0.01 \text{ mg/g}$ whilst domestic food wastes inoculated with all microorganisms (CSM) had a mean value of $2.52 \pm 0.02 \text{ mg/g}$ (Figure 2).

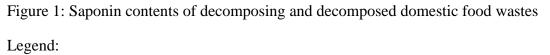
All the test samples showed decrease in tannin contents. The domestic food waste M and E inoculated with *Bacillus sphaericus* + *Macromonnas mobilis* and *Bacillus megaterium* + *lactobacillus jensenii* respectively had the lowest tannin contents of $2.11 \pm 0.02\%$ and $2.42 \pm$

0.01% respectively at week 6 (Figure 3) as compared to sample P which had the highest tannin throughout the composting process with a mean value of $7.60 \pm 0.01\%$ at week 6. The CSM, CC and CD samples showed degradation of tannin throughout the degradation period of 0-6 weeks, with CSM having the least value of $1.82 \pm 0.02\%$.

Generally all the treatments showed a reduction in phytate contents (Figure 4). At 6 weeks, DFW with sample I (*Azotobacter* and *Penicillium italicum*) had the phytate content of 12.01 ± 0.01 mg/g followed by domestic food wastes inoculated with M having a mean value of 11.38 ± 0.01 mg/g. The CSM exhibited the lowest phytate content of 2.55 ± 0.01 mg/g at week 6, followed by CD and CC with mean values of 8.02 ± 0.02 mg/g and 5.39 ± 0.02 mg/g respectively.

All the treated domestic food wastes (DFW) samples had low cyanide contents except for CD which showed mean values of $4.40 \pm \text{mg}/100\text{g}$ and I $1.60 \pm \text{mg}/100\text{g}$ at 0 and 6 weeks respectively representing highest comparative values (Figure 5).





CD: Domestic food waste from Akure main dumpsite

CC: Constituted Domestic food wastes

CSW: Unsterilized domestic food wastes uninoculated with microorganisms

CSM: Sterilized domestic food wastes inoculated with microorganisms.

T: Domestic food wastes inoculated with *Lactobacillus delbrueckii* and *Geobacillus stearothermophilus*

E: Domestic food wastes inoculated with Bacillus megaterium and Lactobacillus jensenii.

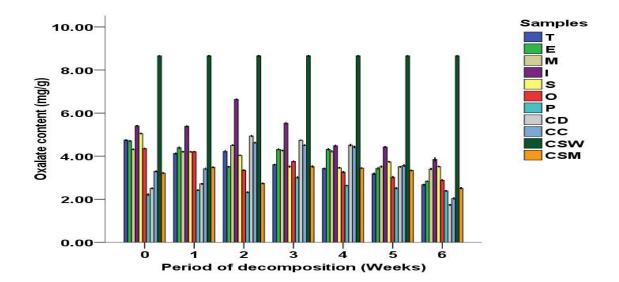
M: Domestic food wastes inoculated with Bacillus sphaericus and Macromonnas mobilis

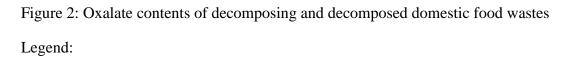
I: Domestic food wastes inoculated with Azotobacter and Penicillium italicum

S: Domestic food wastes inoculated with Listeria monocytogenes and Aspergillus niger

O: Domestic food wastes inoculated with Kurthia species and Aspergillus niger

P: Domestic food wastes inoculated with Varicosporium elodeae and Rhizopus nigricans





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E: Domestic food wastes inoculated with Bacillus megaterium and Lactobacillus jensenii.

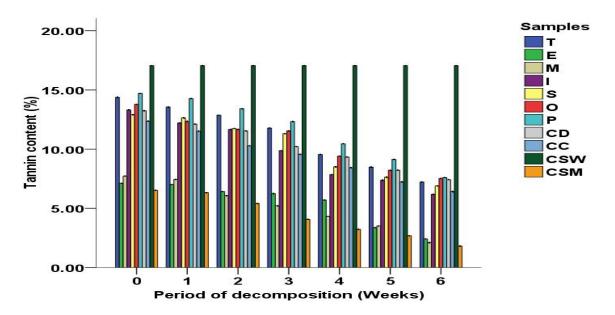
M: Domestic food wastes inoculated with Bacillus sphaericus and Macromonnas mobilis

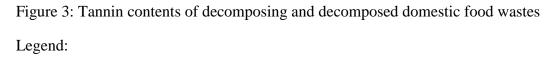
I: Domestic food wastes inoculated with Azotobacter and Penicillium italicum

S: Domestic food wastes inoculated with Listeria monocytogenes and Aspergillus niger

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T: Domestic food wastes inoculated with *Lactobacillus delbrueckii* and *Geobacillus stearothermophilus*

E: Domestic food wastes inoculated with Bacillus megaterium and Lactobacillus jensenii.

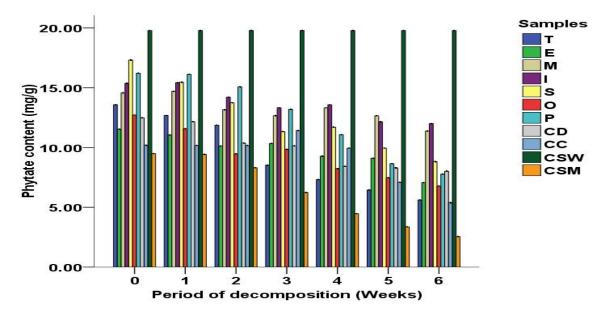
M: Domestic food wastes inoculated with Bacillus sphaericus and Macromonnas mobilis

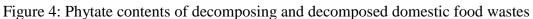
I: Domestic food wastes inoculated with Azotobacter and Penicillium italicum

S: Domestic food wastes inoculated with Listeria monocytogenes and Aspergillus niger

O: Domestic food wastes inoculated with Kurthia species and Aspergillus niger

P: Domestic food wastes inoculated with Varicosporium elodeae and Rhizopus nigricans





Legend:

CD: Domestic food waste from Akure main dumpsite

CC: Constituted Domestic food wastes

CSW: Unsterilized domestic food wastes uninoculated with microorganisms

CSM: Sterilized domestic food wastes inoculated with microorganisms.

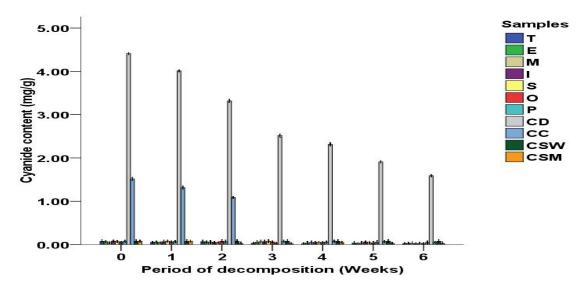
T: Domestic food wastes inoculated with *Lactobacillus delbrueckii* and *Geobacillus stearothermophilus*

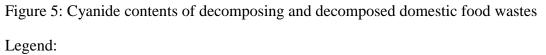
E: Domestic food wastes inoculated with Bacillus megaterium and Lactobacillus jensenii.

M: Domestic food wastes inoculated with Bacillus sphaericus and Macromonnas mobilis

- I: Domestic food wastes inoculated with Azotobacter and Penicillium italicum
- S: Domestic food wastes inoculated with Listeria monocytogenes and Aspergillus niger
- O: Domestic food wastes inoculated with Kurthia species and Aspergillus niger

P: Domestic food wastes inoculated with Varicosporium elodeae and Rhizopus nigricans





- CD: Domestic food waste from Akure main dumpsite
- CC: Constituted Domestic food wastes

CSW: Unsterilized domestic food wastes uninoculated with microorganisms

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T: Domestic food wastes inoculated with *Lactobacillus delbrueckii* and *Geobacillus stearothermophilus*

E: Domestic food wastes inoculated with Bacillus megaterium and Lactobacillus jensenii.

M: Domestic food wastes inoculated with Bacillus sphaericus and Macromonnas mobilis

I: Domestic food wastes inoculated with Azotobacter and Penicillium italicum

S: Domestic food wastes inoculated with Listeria monocytogenes and Aspergillus niger

O: Domestic food wastes inoculated with Kurthia species and Aspergillus niger

P: Domestic food wastes inoculated with Varicosporium elodeae and Rhizopus nigricans

4. Discussions

Antinutrients are natural or synthetic compounds that interfere with the absorption of nutrients. Many traditional methods of food preparation such as fermentation, cooking, and malting increase the nutritive quality of plant foods by reducing the contents of certain antinutrients such as phytic acid, polyphenols, and oxalic acid (Bossert and Bartha, 2010).

Saponins are plant glycosides that derive their name from their soap-like properties. They occur in a great many plant species, and have been implicated as pre-formed determinants of resistance to fungal attack. Some of the constituents of the domestic food waste used were naturally foam producing, which explains why the samples were high in saponin. As saponins haemolyse red blood cells, its elimination by heat makes it safe for human consumption. The reduction in the saponin content indicates that these organisms were able to produce enzymes that degraded saponin. According to Diaz (2008) *Aspergillus niger* has been found to produce beta-tomatinase that degraded saponin.

Oxalate is a very simple sort of molecule. It links up with calcium and crystallizes under some conditions, including when it encounters damaged tissues. Plants use oxalate to protect themselves from infection or from being eaten (Environmental Consultants Inc., 1990). According to Anonymous (2005a) the main oxalate degrading bacteria is *Oxalobacter formigenes*, but *Lactobacillus acidophilus* deprived of its usual food, may be able to "eat" oxalate. The low oxalate content of sample "*Varicosporium elodeae* + *Rhizopus nigricans*" throughout the test indicates that fungal are good degraders of oxalate, *Lactobacillus* species are also degraders of oxalate when the level of nutrients are low, this is evident in samples T (*Lactobacillus delbrueckii* and *Geobacillus stearothermophilus*. and E (*Bacillus megaterium* and *Lactobacillus jensenii*). The degradative activity of *Kurthia* species + *Aspergillus niger*" could

be attributed to the fact that the relationship between both microorganisms is beneficial to each other (Bennett, 2010).

Phytic acid (known as inositolhexakisphosphate (IP6), or phytate when in saltform) is the principal storage form of phosphorus in many planttissues, especially bran and seeds. One common example is phytate, which form insolublecomplexes with calcium, zinc, iron and copper. The low phytate contents in DFW samples decomposed with *Lactobacillus delbrueckii* + *Geobacillus stearothermophilus, Kurthia* species+ *Aspergillus niger* and *Bacillus megaterium* + *Lactobacillus jensenii*indicates that these microorganisms are good degraders of phytate. According to Hurrell (2003) probiotic lactobacilli, are an important source of the enzyme phytase which catalyses' the release of phosphate from phytate and hydrolyses the complexes formed by phytate and metal ions or other cations. The difference in the phytate contents of decomposing and decomposed DFW were sterilized and non-sterilized samples shows that high temperature from autoclaving caused the reduction in the phytate content.Phytic acid and oxalic acid usually forms insoluble salts with mineral element such as zinc, calcium and iron to prevent their utilization (De Angelis *et al.*, 2003).

Tannin affects the digestive tract and their metabolites are toxic (Cupples *et al.*, 2005). The low tannin contents in DFW samples inoculated with *Bacillus sphaericus* + *Macromonnas mobilis* (M) and *Bacillus megaterium* + *Lactobacillus jensenii* (E) shows that these organisms were able to degrade tannin. These microorganisms are able to utilize tannin as part of their sole carbon and energy source (Deschamps *et al.*, 1980; Gandhi, 1990). The ability of microorganisms to degrade tannins has been attributed to the production of tannase, an important enzyme capable of catalyzing gallotannins to garlic acid and glucose. According to Mingshuet al. (2006) *Bacillus* species are able to produce tanneses the enzyme involved in the catabolism of tannin. The result in these samples also shows that the interaction between the mixed organisms could have been beneficial to *Bacillus* spp. The high tannin content in sample P (*Varicosporium elodeae* and *Rhizopus nigricans*) could be as a result of the biomass-specific activities of most enzymes being higher in bacteria than in fungi.

Cyanide upon breakdown release the toxic compound hydrogen cyanide (HCN) which can be harmful to the consumers through an enzyme catalyzed process called cyanogenesis, cyanide (hydrocyanic acid) can be produced through enzymatic process which occurs when the plant cells are bruised, crushed, grated or bitten and when cyanogen's and degradative enzyme come in contact with each other (Cunningham et al., 1996). Generally the samples were low in cyanide concentrations. The CD sample had the highest cyanide content showing that it contained materials having the antinutrient or contained cyanide producing bacteria: the reduction in the cyanide content could be due to the presence of cyanide degrading microorganisms. The microorganisms were able to degrade cyanide to a level of non-toxicity to plants or humans. For hydrogen cyanide the fatal dose in food is 50mg/100g which is higher than what was obtained in the Compost sample (2.8mg/100g). Such illnesses arising from its excesses cyanide like gasping, staggering, paralysis convulsion could be avoided. The difference in the concentrations of treated and non-treated samples shows that temperature caused the cyanide reduction.According to Anonymous (2003) Bacillus sphaericus and *Geobacillus* stearothermophilus produce the enzyme Rhodanese in minute quantities that could degrade cyanide as the sole carbon and nitrogen source, by formate dehydrogenase. Antinutrients can be reduced during cooking, fermentation and soaking. This confirms the report of other researchers (Aregheore 1998) that cooking and fermentation do indeed destroy antinutritional factors. (Gilani *et al.*, 2005).

5. Conclusions

The efficient degradation of the antinutrient contents of the domestic food wastes shows that these organisms can be used in preparing compost from domestic waste therebyreducing the volume of garbage needlessly sent to landfills. The anti-nutrient contents were reduced in the presence of the test micro faunas. The stabilized end-product (compost) can be widely used as a soil amendment to improve soil structure and provide plant nutrients. It is recommended that the combination of these microorganisms be used to prepare compost on a large scale.

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