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Comparative Microbial Analysis of Earthworm Casts Collected From Ikenne, Ogun State, Nigeria

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Abstract

A comparative assessment of the physicochemical parameters and microbial profile of three types of earthworm casts (Pellet, Mass and Turret) were determined. The total viable count (TVC), coliform counts, yeast and mould counts were determined using standard procedures. The pH ranged from 7.8 for Mass cast to 8.6 for Pellet cast. Moisture content of the casts ranged from 29.43% for pellet casts to 47.10% for turret casts. Organic matter was 1.05%, 0.72% and 0.63% for pellet, turret and mass casts respectively. TVC was 4.8 x 10^7cfu/g, 6.1 x 10^7cfu/g and 1.2 x 10^8cfu/g for Mass, Turret and Pellet casts respectively. Pellet cast recorded the highest coliform count (1.5 x 10^7) while Turret cast the least (7.3 x 10^6 cfu/g). Mould counts was 8.3 x 10^6 cfu/g for turret cast and 1.2 x 10^7cfu/g for pellet and mass casts. The lowest yeasts count was 1.2 x 10^7cfu/g in mass cast while the highest was 3.8 x 10^7cfu/g for pellet cast. Microbial distribution in the three casts types showed that Staphylococcus aureus is common to all Cast-types. Citrobacter spp, Pseudomonas fluorescens, Penicillium chrysogenum and S. rosei were isolated from Pellet cast only. Aspergillus fumigatus, Fusarium oxisporum, Pseudomonas aeruginosa and Penicillium oxalicum were isolated from only Turret Cast while Aspergillus terreus, Fusarium compacticum, Klebsiella aerogenes and Streptococcus feacalis were isolated from Mass Cast only. The presence of Bacillus licheniformis in Pellet Cast is an advantage plus for Pellet cast usage since this bacteria contributes to nutrient cycling and displays antifungal activities.

Keywords: Pellet cast, Turret cast, Mass cast, Microbial Count,

Introduction

Casting by earthworm is an important activity which, have been shown by several studies to have significant impact on soil fertility. A worm casting is a biologically active mound containing several bacteria, enzymes and remnants of plant materials and animal manure that were not digested by the earthworm (Appelhof, 1982). Earthworms and their casts are useful in land improvement, reclamation and in organic waste management (Edwards and Baker 1992; Johnson 1997; Lavelle and Martin 1992; Villenave *et al.* 1999).

Studies have revealed that earthworms contribute significantly to soil structure and soil fertility, improve water absorption and crop yield. (Edwards and Lofty, 1977; Abbot and Parker, 1981; Owa *et al.,* 2003; Owa *et al.,* 2004a, 2004b). Edwards and Bates (1992) reported that earthworms increased significantly the number, growth rate and yield of plants on inoculated sites. Logsdon (1994) showed that earthworms successfully decomposed sugar factory residuals and turned them into soil nutrient that allowed reduction in the use of chemical fertilizers by 50%. Earthworms have been shown to increase soil porosity and soil aeration and bring about decomposition of leaf and other litters, thereby making soil nitrates and phosphorus more available in the soil (Owa *et al.*, 2003).

Earthworm casts significantly affect plant growth through their effects on microorganisms, aggregation of soil, and nutrient supply (Sabrina *et al.,* 2009). Reports have shown that enzymes such as β-glucosidase, alkaline phosphatase, dehydrogenase and protease are more abundant in the castings than the surrounding soil (Aira *et al*, 2004). Castings has been shown to absorb water faster than soil and hold more water than equivalent amounts of soil thereby increasing moisture absorption and moisture availability to plants. Furthermore, castings are known to absorb moisture from the air and hold it for plant use. Casting also holds nutrients for plant use as a non-pollutant natural fertilizer. An important component of casting is humic acid, which provides binding sites for plant nutrients such as calcium, iron, potassium, sulfur and phosphorus and releases them on demand to the plants (Sabrina *et al.,* 2009). Casts have been shown to have enhanced microbial and enzyme activities and micro- and macro-nutrients (Vinotha *et al.,* 2000).

In addition, nitrogen fixing bacteria which are of earthworm gut origin had been found in the castings. This resulted in higher nitrogenase activity in earthworm castings and hence greater rates of nitrogen fixation compared to the surrounding. Several reports found that castings generally have a higher ammonium concentration than bulk soils and

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they constitute sites of high denitrification potential (Elliot *et al,* 1990).

Logsdon (1994) showed that microbial activity in worm castings is 10-20 times higher than the soil and initial organic material ingested by the earthworm. This observation was further confirmed by Card *et al* (2004). This has contributed to the enrichment of the soil because the intestine of earthworms serving as a micro-incubator provides a conducive environment, within which ingested microbes could multiply rapidly. Hence, earthworms and their enteric microbes play a major role in pedogenesis, soil aggregate formation and soil aeration (Feller *et al.,* 2003).

Three major cast-types were observed to be produced by earthworms namely: Pellet casts (granular casts) produced by *Eudrilus eugeniae, Agrotoreutus spp* and *Eutoreutus spp* (Sims, 1971; Segun, 1976); Turret casts (funnel/finger shaped) produced by *Hyperiodrilus africanus* and *Ephyriodrilus afroccidentalis* and the Mass (moldy) casts produced by *Libyodrilus violaceus* (Sims, 1971; Beddard, 1981).

Though there have been studies which showed that earthworm castings encourage high proliferation of microflora but most of these past works lumped all cast-types together, hence, no established data on the relative microbial population in different earthworm cast-types. This work therefore is aimed at establishing the relative microbial population of pellet, turret and mass cast-types collected from Ikenne Campus of Olabisi Onabanjo University, Ogun State.

Study area

Cast samples were collected from two sites within the Olabisi Onabanjo University, Ikenne Campus, Ogun State. Ikenne is located in South-West geo-political region of Nigeria on latitude $6^0$52'N and longitude $3^0$43'E in the rainforest zone with a mean annual rainfall of 1100mm. The major soil type is sandy-loam (top soil) to sandy-clay (subsoil) and the major food crops include maize, cassava, yam, rice, water melon, pineapple, cocoyam, cowpea and vegetables while the major cash crops include cocoa, rubber and cashew, oil palm tree, kolanut and timber.

Materials and Methods

Cast collection:

Turret casts were collected from under hedgerows within the University Campus, while the Mass and Pellet Cast were collected from more open lands located few paces away from the hedgerows. The different cast samples were collected into pre-labeled polythene bags and transferred to the soil laboratory for analysis.

Physicochemical Analysis of Casts

The pH of the casts was measured using Mettler digital pH meter with probe. The moisture content of casts was determined by the conventional method of finding the difference of fresh casts versus oven dried casts. The Organic Carbon and Organic Matter were determined by the Walkey and Black method (1934). available Phosphorus in the cast was analysed using the Bray 2 method (Bray and Kurtz, 1945). The phenol-disulphonic acid colorimetric method was used to determine the % Nitrate of the Castings. The total Nitrogen was determined using the Kjeldahl method (Bremner, 1960);

Microbial Cultivation and Enumeration.

Ten-fold dilutions of each of the samples were made using peptone water. Appropriate dilutions were made and 0.1 ml of the diluted samples were pour plated in triplicate plates on Plate Count Agar (PCA) for viable count, Eosin Methylene Blue (EMB) for *Escherichia coli* count, Mannitol Salt Agar (MSA) for *Staphylococcal* count and Brilliant Green Bile Broth (BGBB) for coliform test. Sabourand Dextrose Agar with Chloramphenicol (250mg/100ml) was used for fungi, while for yeast count the medium was adjusted to pH 3.5 with tartaric acid. All plates were incubated for 48 hours at 30°C except for Sabourand Dextrose Agar that were incubated at 25°C for 6 days. Pure cultures of each isolate were obtained by streaking the specific colonies on suitable media and incubated appropriately, these were maintained in an agar slants in McCartney bottles.

Identification of microbial isolates

The identification of the bacteria colonies was based on classification schemes proposed by Harrigan and McCance (1976), Buchanan and Gibbons (1974) and Collins and Lyne, (1984). The identification was based essentially on morphology and biochemical reactions**.** Fungi genus were determined through morphological criteria using identification keys such as the description of mycelia and of asexual reproduction forms (Domsch *et al.,* 1980).

Data Analysis:

The results were subjected to statistical analysis, these include Descriptive statistics,

Analysis of Variance (ANOVA), Duncan Multiple Range test, Bivariate Pearson Correlation using the Statistical Package for the Social Science (SPSS) version 11.0 was used for these analysis.

Results

Table 1 presents the physicochemical parameters of different earthworm casts examined. The pH ranged from 7.8 for Mass cast to 8.6 for Pellet cast. The highest moisture content observed was 47.10% for Turret cast, followed by 45.60% for Mass cast and 29% for Pellet cast.

The Statistical analysis as confirmed by the Duncan multiple range test (Table 3) showed that there were significant differences between the means of all the physicochemical parameters studied. With the exception of the %Moisture content, the mean values of all other parameters were significantly ($p<0.05$) higher for Pellet casts as compared to Mass and Turret casts.

The mean microbial counts in the different cast-types presented in Table 2 showed Total Viable Count (TVC) of 4.8×10^7 cfu/g, 6.1×10^7 cfu/g and 1.2×10^8 cfu/g for Mass, Turret and Pellet casts respectively. Coliform count ranged from 7.3×10^6 cfu/g for Turret cast to 1.5×10^7 cfu/g for Pellet cast. Mould counts was 8.3×10^6 for turret cast and 1.2×10^7 cfu/g for pellet and mass casts. The lowest yeasts count was 1.2×10^7 cfu/g in mass cast while the highest was 3.8×10^7 cfu/g for pellet cast.

Generally significantly ($p<0.05$) higher microbial count was observed for Pellet cast as compared to the other cast-types examined. Statistical analysis also showed that the differences between the mean microbial counts were significant (Table 4).

The distribution of the microbial isolates in the different earthworm cast-types is presented in Table 5. Fourteen (14) bacterial species isolated from the three casts were *Aerobacter aerogenes, Bacillus cereus, Bacillus licheniformis, Bacillus cereus, Citrobacter spp, Escherichia coli, Klebsiella aerogenes, Proteus vulgaris, Proteus morgani, Pseudomonas fragii, Pseudomonas aeruginosa, Pseudomonas fluorescens, Staphylococcus aureus, Streptococcus faecalis* and *Serratia marcescens.* Out of these 15 bacterial species, *Staphylococcus aureus* was obtained from the three casts, *Citrobacter spp* and *Pseudomonas fluorescens* were isolated only from Pellet cast; *Streptococcus faecalis* and *Klebsiella aerogenes* were isolated only from Mass casts while *Pseudomonas aeruginosa* was isolated only from Turret casts. *Bacillus cereus, Pseudomonas fragii* and *Proteus vulgaris* were isolated only from pellet and turret casts and not from any of the mass cast examined.

Seven mould species were isolated from the three casts and they are *Aspergillus niger, Aspergillus fumigatus, Aspergillus terreus, Fusarium compacticum, Fusarium oxisporium, Penicillium chrysogenum* and *Penicillium oxilicum.* Only *Aspergillus niger* and *Penicillium chrysogenum* were isolated from pellet casts; *A. fumigates, F. oxisporium* and *P. oxilicum* were isolated from Turret casts while *A. niger, A. terreus* and *F. compacticum* were obtained from mass casts.

Four yeasts species isolated were *Candida spp, Saccharomyces rosei, S. cerevisiae* and *S. bayanus.* Out of the four yeasts species, only *S. cerevisiae* was isolated from mass casts. *S. cerevisiae* was not isolated from turret casts and *S. rosei* was not isolated from pellet cast.

Discussion

The low moisture content of Pellet Cast compared to Turret Cast and Mass Cast suggests that the water holding capacity of turret and mass casts is much higher than Pellet cast. This low water holding capacity of pellet cast may have been due to its tiny granule-like aggregates which may make it susceptible to quicker water loss than the other cast-types**.** The pH of all cast samples fall within the range required for favourable growth of soil bacteria and fungi.

Result of this study showed the presence of several bacterial and fungal species in the different earthworm cast examined. This agrees with the findings of several authors who had reported the occurrence of several species of bacteria and fungi in earthworm casts (Alauzet, *et al.*, 2001; Prakash, *et al.,* 2008; Jayakumar, *et al.,* 2009). Microorganisms generally flourish in earthworm casts (Domsch and Banse 1972). Card *et al.,* 2004 stated that earthworm casts contain many more microbes than its surrounding soil because the intestines of earthworms inoculate the casts with microbes.

The presence of the fungi *Aspergillus*, *Penicillium* and *Fusarium* in the earthworms casts is not surprising as the genera *Aspergillus* and *Penicillium* are frequently found in soils (Torres *et al.*, 1999; Alauzet, *et al.*, 2001).

Tiunov and Scheu (2000) reported that the biomass of fungi is higher in *Lumbricus terrestris* casts than in the surrounding soil. It has also been suggested that the fungi in the casts constitute a nutrient pool for earthworms (Edwards and Fletcher 1988;).

The presence of *Fusarium oxisporum,* a plant pathogen in turret cast and *Klebsiella aerogenes* and *Streptococcus feacalis* in mass cast might have been due to the ingestion of the bacteria species and the spores of this fungus by the earthworm species associated with them. Previous work in literature had earlier suggested that earthworms may ingest the spores of this microorganisms and deposits it along with their casts (Edwards and Lofty, 1977; Alauzet, *et al.*, 2001) The presence of *Bacillus licheniformis* in Pellet Cast is an added advantage to this cast since these bacteria contribute to nutrient cycling and displays antifungal activities.

Furthermore, the higher pH, organic matter, organic carbon, % Nitrite, % Nitrate, % Nitrogen and % Phosphorus are probable the contributive factors leading to the higher Total Viable Count (TVC) observed in Pellet cast as confirmed by the Bivariate Pearson Correlation and this may have offset the negative impact of its low moisture content. This is in agreement with Atlas (1997) who revealed that these energy and electron sources (the organic and inorganic constituents of the casts) are required for microbial proliferation and vice versa.

The importance of these microbial population in earthworm casts cannot be overemphasized, their presence and bioactivities in the cast in larger amount than the surrounding soil resulted in higher organic matter, carbon content, phosphate, nitrate and nitrogen content than their surrounding soil. This makes earthworm casts suitable media for raising seedling in horticultural practices and the boost of nutrient in the cast will produce better seedling performance. Furthermore, the cast types serve as reservoir from which microbes could be transported and inoculated to new or reclaimed soil as reported by Edwards and Lofty (1977).

In conclusion, this study has shown that there is significant difference between the nutrient content, viable microbial count (load), Coliform Count, Fungal Count and Yeast Count of Pellet, Turret and Mass Casts. There is a significant and positive relationship between the microbial loads, organic matter and inorganic constituents of Pellet, Turret and Mass Casts with Pellet Cast containing higher amount of all these parameters hence will contribute more nutrient to enhance soil fertility.

However, this notwithstanding, all the cast-types could be said to play a synergistic role in enhancing soil fertility with pellet cast serving as a quick release nutrient capsules for early germination of crops before the onset of the rainy season whereas turret and mass casts would serve as a long time release capsule of nutrients during the later periods of the rainy season. It is therefore advisable for farmers and horticulturist in this area to encourage the presence of different species of earthworms, the turret casters, mass casters and pellet casters in the soil.

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Table1: Descriptive Statistics of the physicochemical parameters of the Earthworm casts

Cast-types	*Mean and Std. Dev.*	*pH*	*% moisture content*	*%Organic Carbon*	*%Organic matter*	*% Phosphate*	*% Nitrate*	*% total Nitrogen*
Pellet Cast	Mean	8.60	29.43	0.61	1.05	0.04	0.02	0.06
	N	5	10	3	3	3	3	3
	Std. Dev.	0.01	0.08	0.01	0.02	0.001	0.001	0.001
Turret Cast	Mean	8.09	47.10	0.42	0.72	0.03	0.01	0.05
	N	5	10	3	3	3	3	3
	Std. Dev.	0.01	0.14	0.01	0.02	0.001	0.00	0.001
Mass Cast	Mean	7.81	45.60	0.36	0.63	0.02	0.02	0.05
	N	5	10	3	3	3	3	3
	Std. Dev.	0.01	0.09	0.01	0.01	0.001	.001	0.00
Total	Mean	8.17	40.71	0.46	0.79	0.03	.02	0.06
	N	15	30	9	9	9	9	9
	Std. Dev.	0.34	8.14	0.11	0.19	0.01	0.002	0.01

Table 2: Descriptive Statistics of Microbial load of Earthworm casts

Cast-types	*Mean and Std. Dev.*	*Total Viable Count*	*Coliform Count*	*Mould Count*	*Yeast Count*
Pellet Cast	Mean	1.20×10^8	1.5×10^7	1.2×10^7	3.8×10^7
	N	3	3	3	3
	Std. Dev.	0.58	0.58	0.58	1.00
Turret Cast	Mean	6.1×10^7	7.3×10^6	8.3×10^6	2.8×10^7
	N	3	3	3	3
	Std. Dev.	1.00	0.58	0.58	1.00
Mass Cast	Mean	4.8×10^7	1.0×10^7	1.2×10^7	1.2×10^7
	N	3	3	3	3
	Std. Dev.	1.00	0.58	1.00	0.58

Table 3: Duncan Multiple Comparison of the physicochemical parameters of the Earthworm casts.

Parameter	*Cast-types*		
	Mass Cast	Turret Cast	Pellet Cast
pH	7.81^c	8.09^b	8.60^a
% Moisture Content	45.60^b	47.10^a	29.43^c
% Organic Carbon	0.36^c	0.42^b	0.61^a
% Organic Matter	0.63^c	0.72^b	1.05^a
% Phosphate	0.02^c	0.03^b	0.04^a
% Nitrate	0.015^b	0.013^c	0.018^a
% Nitrogen	0.054^b	0.048^c	0.063^a

Means with the same superscript in a row are not significantly different

Table 4: Duncan Multiple Comparison of the Microbial load of Earthworm casts

Parameter	***Cast-types***		
	Mass Cast	**Turret Cast**	**Pellet Cast**
Total Viable Count	48.00[c]	61.00[b]	120.33[a]
Coliform Count	10.33[b]	7.33[c]	15.33[a]
Mould Count	12.00[a]	8.33[b]	12.33[a]
Yeast Count	12.33[c]	28.00[b]	38.00[a]

Means with the same superscript in a row are not significantly different

Table 5: Distributions of isolates in the different cast-types

S/N	**Isolates**	**Pellet Cast**	**Turret Cast**	**Mass Cast**
	Bacterial			
1.	*Aerobacter aerogenes*	-	+	+
2.	*Bacillus cereus*	+	+	-
3.	*Bacillus licheniformis*	+	-	+
4.	*Citrobacter spp*	+	-	-
5.	*Escherichia coli*	+	-	+
6.	*Klebsiella aerogenes*	-	-	+
7.	*Proteus vulgaris*	+	+	-
8.	*Proteus morgani*	+	-	+
9.	*Pseudomonas fragii*	+	+	-
10.	*Pseudomonas aeruginosa*	-	+	-
11.	*Pseudomonas fluorescens*	+	-	-
12.	*Staphylococcus aureus*	+	+	+
13.	*Streptococcus faecalis*	-	-	+
14.	*Serratia marcescens*	+	-	+
	Mould			
15.	*Aspergillus niger*	+	-	+
16.	*Aspergillus fumigatus*	-	+	-
17.	*Aspergillus terreus*	-	-	+
18.	*Fusarium compacticum*	-	-	+
19.	*Fusarium oxisporium*	-	+	-
20.	*Penicillium chrysogenum*	+	-	-
21.	*Penicillium oxilicum*	-	+	-
	Yeast			
22.	*Candida spp*	+	+	-
22.	*Saccharomyces rosei*	+	-	-
23.	*Saccharomyces cerevisiae*	-	+	+
24.	*Saccharomyces bayanus*	+	+	-