



Evolution of the Dominant Groups of Micro-organisms during the Composting Process of the Household Refuses from the Dschang Municipality – Cameroon

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Authors' contributions

This work was carried out in collaboration between all authors. Authors NE and TE designed the study, wrote the protocol. Authors NTH and KKGS managed the analyses of the study, managed the literature searches. Author KKGS conducted field and lab researches. Author TE wrote the first draft of the manuscript. All authors read and approved the final manuscript.

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ABSTRACT

Aims: This study was aimed at identifying and characterizing the different microbial groups that are involved in the composting process of household waste from the city of Dschang.

Study Design: Three compost heaps of household wastes were studied, and a composite sample was collected once every two weeks during the four months period for microbiological analysis.

Place and Duration of Study: The Household Waste Composting Pilote Project of the Dschang

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municipality, during the four months study period.

Methodology: The total bacterial and fungal microflora have been identified according to the suspensions-dilutions technique on a specific solid medium, incubated and then counted. The confirmation test for fungi was the methylene blue.

Results: Three major groups of microorganisms were present in the compost from household waste from the Dschang municipality that we studied: Bacteria, Actinomycetes and Fungi. The density of bacteria decreases with the phases of the composting while actinomycetes and fungi are increasing. Among the microbial strains identified, some are present in all the composting phases (*Pseudomonas*, *Penicillium*, *Rhizopus* and *Nocardia*), others disappear in the cooling phase (*Mucor* spp) and others finally appear only at the phase of maturation (*Saccharomycetes*).

Conclusion: The evolution of fungal and bacterial diversity during the composting process is parallel to that of their density because the maintenance, the disappearance or appearance of certain strains can be explained by the variation in the temperature, moisture, pH, biodegradable organic matter and competitions between the microbial strains.

Keywords: Household waste; composting; bacteria; Actinomycetes; fungi; Dschang municipality.

1. INTRODUCTION

The management of household waste is classified among the priority conditions for sustainable urban development [1]. Ngnikam and Tanawa [2] emphasized the importance of the fermentable fraction in household waste for the countries of the south. One of the most interesting in the valuation of organic material is composting which encompasses the aerobic biodegradation of wastes into a more stable organic product rich in humic compounds called compost. According to several authors [3-5], composting is a process of biological transformation of various organic materials into humus by the action of a large number of microorganisms in a warm, moist, warm and airy environment. The products formed are mainly CO₂ and a stabilized product which is the mature compost. The organic waste at the beginning is colonized, transformed by a succession of different microbial populations. Each of these populations modifies the environment and is then replaced by others better adapted to the new conditions. Different communities of microorganisms, consisting mainly of bacteria, fungi and protozoa follow each other during this process [6-8]. The rise of the temperature allows a selection of resistant germs which will survive the thermal peak. During cooling, the window is re-colonized by another group of microorganisms that will promote the humification [9]. In fact the composting of urban waste also offers very interesting solutions to transform the organic waste into a resource. The scientific work which are interested in composting are looking much more on the quality of the compost, its chemical composition and its agronomic value [10-12]. But the aspect concerning its microbial diversity -

"real engineers" - of the process and the various modes of establishment of these microorganisms remains unexplored. If it is true that several microbial groups are involved in the decomposition of waste throughout the duration of the composting process, knowledge of the various groups of microorganisms which react at different phases of composting still remains less clarified. The general objective of this work is thus to determine the evolution of the microbial communities of the marketable compost from the household waste of the town of Dschang-Cameroun. Specifically, it is to follow the installation of microbial populations during the process of composting and during different phases; to characterize the various groups of micro-organisms and to evaluate the microbial group.

2. MATERIALS AND METHODS

2.1 Site Description and Localization

The study was carried out from April 28 to August 28, 2012 in the Ngué discharge of the city of Dschang. Dschang is the head quarter of the Menoua Division, Western Region of Cameroun. The city is located at 200 kilometers from Douala, the economic capital and at 350 kilometers from Yaoundé, the political capital of Cameroun. It is situated on the South-eastern slope of the Mount Bamboutos (1000 to 1600 meters above sea level), which culminates at 2740 m above sea level, and has an old base covered with volcanic formations. With its geographical co-ordinates (5°25'-5°30' Northern Latitude and 10°-10°5' Eastern Longitude) (Fig. 1), Dschang is thus in the subequatorial zone:

the rainy season extends from March to October and the dry season from November to February. Temperatures vary from 13°C to 28°C, with an average of 20.5°C. The average precipitations are 1900 mm/y. This climate makes the area the most significant agricultural zone of the country [13], and agriculture represents about 50% of the employment sector.

2.2 Manufacture of Compost

After selection of the household waste collected in the city by separation of the non-fermentable fraction for discharge, the fermentable fraction was kept for the formation of composting heaps. Three heaps were thus formed, with 1.5 m height at the base and 2 m width, all having conical form to permit good ventilation. The daily temperature measurement was used to determine the periods to turn our compost because according to [2], a decrease of temperature by 10 degree is enough to turn and aerate the heap in composting for the first four reversals and of 5 degree for the fifth and so on.

After maturation, the dried and sieved compost was stored in 50 kg bags.

2.3 Sampling

Each composite sample was made from five different ways on each of the heaps, either during overturning of the pile, or after opening a vertical bleeding so as to obtain sample as representative as possible. The technique recommended by [10,14] was used to collect representative sample. Sampling lasted four months, and samples of 0.5 kg each, were taken of the evaluation and identification of the fungal in safe containers every two weeks. The microbiological analysis consisted and bacterial flora and actinomycetes. A few physico-chemical parameters analyzed for the purpose (pH, organic carbon, nitrogen, exchangeable cations, etc.) were the subject of other publication [12]. Table 1 indicates the periods or phases of sampling. For this study, three phases only were considered, given that the mesophilic phase went on unnoticed.

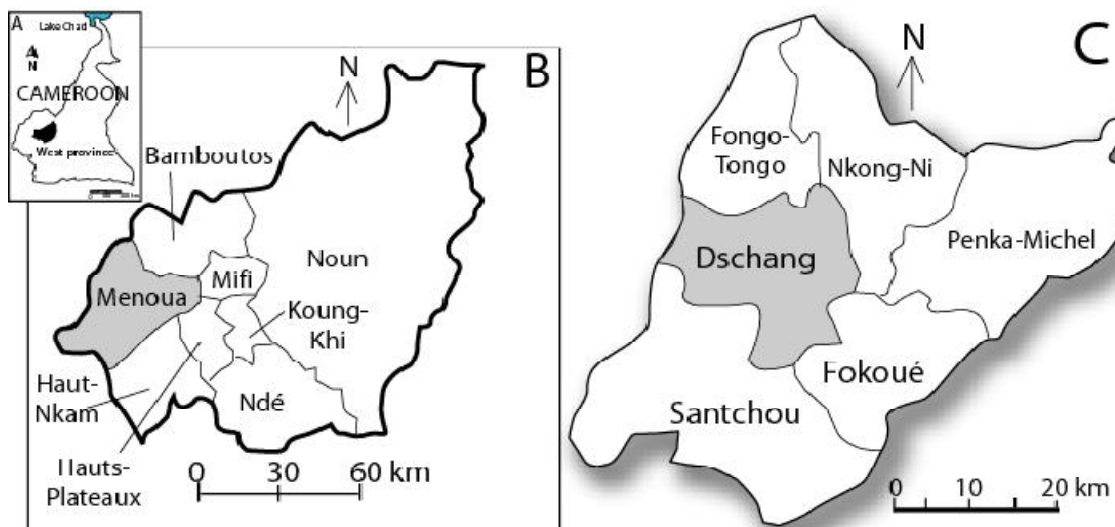
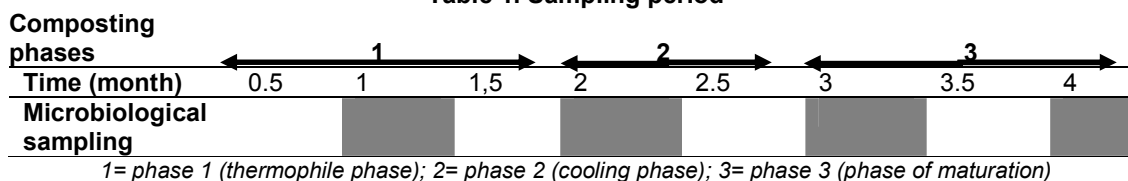


Fig. 1. Localization of the city of Dschang in the Menoua division (C), in the West Region (B) and in Cameroun (A) [12]

Table 1. Sampling period



2.4 Microbiological Analyses

Micro-organisms were evaluated and identified according to the technique described by [15].

2.4.1 Fungal microflora

The suspension-dilutions technique was used for the culture of fungi on the specific solid medium of sabouraud added to chloramphenicol (sigma) to 0.005%. To do this, one gram of sample previously well homogenized was suspended in 10 ml solution (1.2g of bactopectone, 6 g of sodium pyrophosphate in 1000 ml of sterilized and distilled water). After 30 minutes of agitation and 20 minutes of settling, decimal dilutions were performed from this suspension. 0.1 ml of each dilution was inoculated in the petri dish using sterile balls of glass at a rate of 5 repetitions per dilution. The sown boxes were thus incubated in a culture chamber at 30°C. The number of Unit Forming a Colony (CFU) was regularly counted until the fifth day and then different morphotypes were isolated and purified. For each sample, the more numerically represented morphotypes were selected for subsequent studies.

2.4.2 Total bacterial microflora

Five grams of each sample were placed in a sterile erlenmeyer flask of 100 ml containing 45 ml of physiological sterilized water (9 g of NaCl/l of distilled water) and put in suspension using a magnetic stirrer for 30 minutes. The suspension was then made to settle for 20 minutes, and then part of it was taken constituting a dilution of 10^{-1} . From this suspension, other dilutions were made up to 10^{-10} . These dilutions were used to inoculate the culture medium (Nutrient agar) on a petri dish from 0.1 ml of each dilution. All of the boxes were incubated at 30°C and the counting was done two days later.

2.4.3 Isolation of strains of actinomycetes

Actinomycetes were grown in the same culture medium like the fungi (sabouraud chloramphenicol agar). For each sample, 1 g of compost was diluted in 9 ml of physiological sterilized water, and then agitated twice for 5 minutes. This suspension constituted the 10^{-1} dilution. A serie of decimal dilutions (up to 10^{-5}) was thus carried out for each sample. The dishes were incubated at 28°C and observed daily for four weeks. The colonies of actinomycetes were identified according to their macroscopic appearance characteristic (colonies harsh

imprinted into the agar), and by their microscopic appearance (circular colonies consisting of hyphae), following a direct observation under optical microscope (Leica is their Destiny", magnification $\times 10$). For a more clear comment, a whole colony was placed on a sterile blade and after elimination of the maximum of Agar, it was slightly crushed with a coverslip and observed under optical microscope (magnification $\times 40$) [15].

2.4.4 Gram stain

The coloration of Gram was carried out according to the conventional method. The smears of colonies that meet the macroscopic and microscopic characteristics of actinomycetes were prepared, colored and observed under optical microscope (magnification $\times 100$). The actinomycetes are Gram-positive. The confirmation test for fungi was the bleu methylene. After incubation, the readings were taken at 24 h or 48 h for the total microflora and for sporulated bacteria, 3 days for fungi and from 5 days for the actinomycetes.

3. RESULTS AND DISCUSSION

3.1 Characterization of Garbage

The results of the characterization of household garbage showed that for a given mass of garbage deposited on the platform of the site of composting, more than 90 percent of the latter constituted the fermentable fraction whereas the refusal represented only 9.4 percent of the deposit. In the refusal, plastic material, textile and old shoes are more represented with 4.23; 2.84 and 1.06% respectively. These Figures are similar to those of [16,17] who consider 90% of the whole materials valorized in agriculture for the household refuse of urban origin in cities of Ouagadougou and Dschang; they exceed however the average on African cities obtained by [2,18], which is 50-70 percent of the raw material.

3.2 Evolution of the Temperature and Identification of Composting Phases

At the end of the composting which lasted four months, we noticed a variation of temperature in three phases during the exercise. Fig. 2 shows the curve of the evolution of the temperature during composting. From these curves, it appears that temperatures of all the piles had

evolved rapidly to be more than 50°C from the second day of composting. According to some authors [6,19], this corresponds to the mesophilic phase but it was very brief for our study and was not taken into account during sampling. This was followed by an increase of temperature of the pile to a maximum of 75°C which remained constant during ten days: For this study, it was the thermophilic phase (phase 1), which could cause reduction of pathogens and the elimination of the seeds of weeds [20]. The elevation of the temperature at the beginning of composting was due to intensive microbial activity induced by the presence of readily biodegradable organic matter [21]. After the thermophilic phase, we observed a decrease in temperature that stabilized at 42°C and which lasted approximately two and a half months, corresponding to the cooling phase (phase 2). This temperature drop could be explained by a slowdown in the activity of microorganisms due to the depletion of readily degradable organic materials. These observations were also noted by [6,19]. We also noted another phase called phase of maturation after cooling (phase 3), which lasted a month and a half during which the average temperature of

the pile was around 37°C. Jimenez & Garcia [22] also found that the temperature in compost increased during the first few days, up to 60 or 70°C, and then decreased gradually to a constant temperature.

3.3 Evolution of the Rate of Moisture and pH

At the end of 120 days of composting, the moisture rate varied from 55.28 to 70.85 percent in the various heaps (Fig. 3). It increased up to 71 percent from the first week and then decreased to 56.84 percent where it stabilized until the end of the composting. The fluctuations in the rate of moisture observed could be due firstly to the presence of aerobic microorganisms and, secondly to the heterogeneity and the quality of the original substrates (additional peelings of banana, cocoyams, vegetable wastes, maize...). The humidity rate declined under the combined action of the temperature rise and aeration following the rollover which resulted in the loss of water in the form of vapour [6,23].

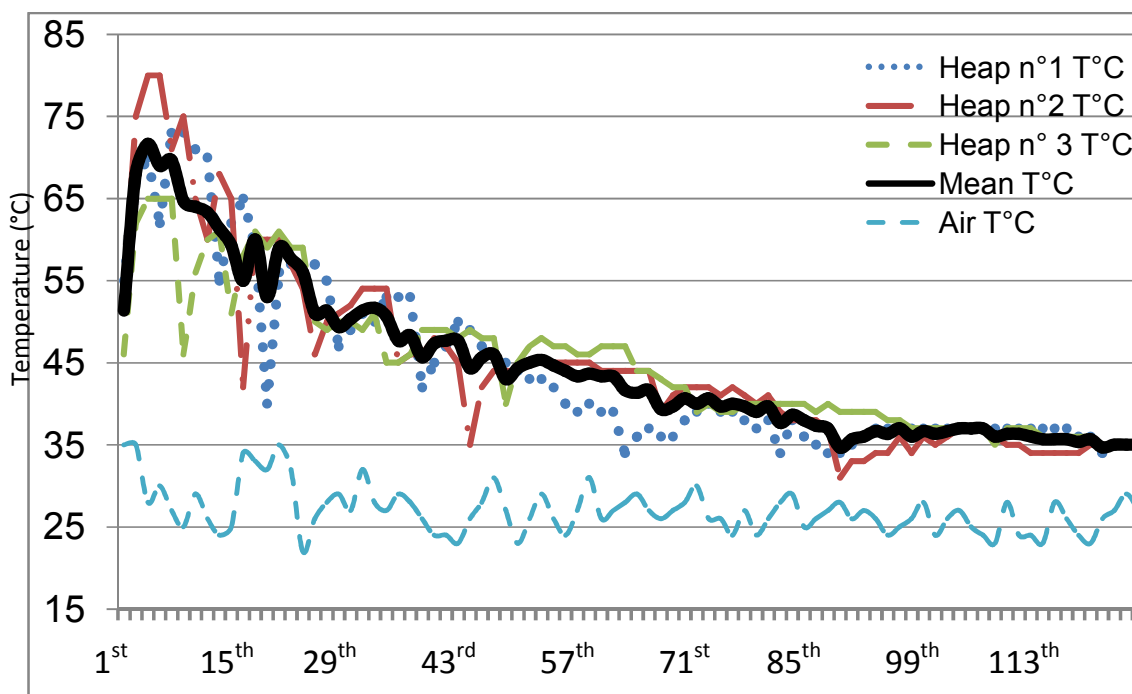


Fig. 2. Change of the temperature during the process of composting

Fig. 4 shows the curve of the evolution of the pH of our compost. It presents the average values in the range between 7.14 and 8.91 in the first weeks, and then a decline to 8.85 hence, stabilizing until the end of the composting. According to [6], this increase in pH is due to the degradation of fatty acids in short chains and to the release of ammonia due to degradation of organic acids and also to ammonium ions NH_4 released during the process. The stabilization of the pH at the end of the process is attributed to the oxidation of ammonium by the bacteria and the precipitation of calcium carbonate [24]. Several other authors [22,25,26] have reported a gradual increase in the pH of the order of 7 to 9 during composting.

3.4 Evolution of Micro-organisms Groups

The composition in microorganisms of the compost studied varied considerably during the different phases of composting (Fig. 5). Three major groups and six strains of microorganisms were present: the group of bacteria having as strain *Pseudomonas* spp, actinomycetes having as strain *Nocardia* and fungi represented by four strains (*Penicillium* spp, *Rhizopus* spp, *Mucor* spp, *Saccharomyces* spp). The bacteria declined with the phases of composting, while actinomycetes and fungi increased with the phases of composting.

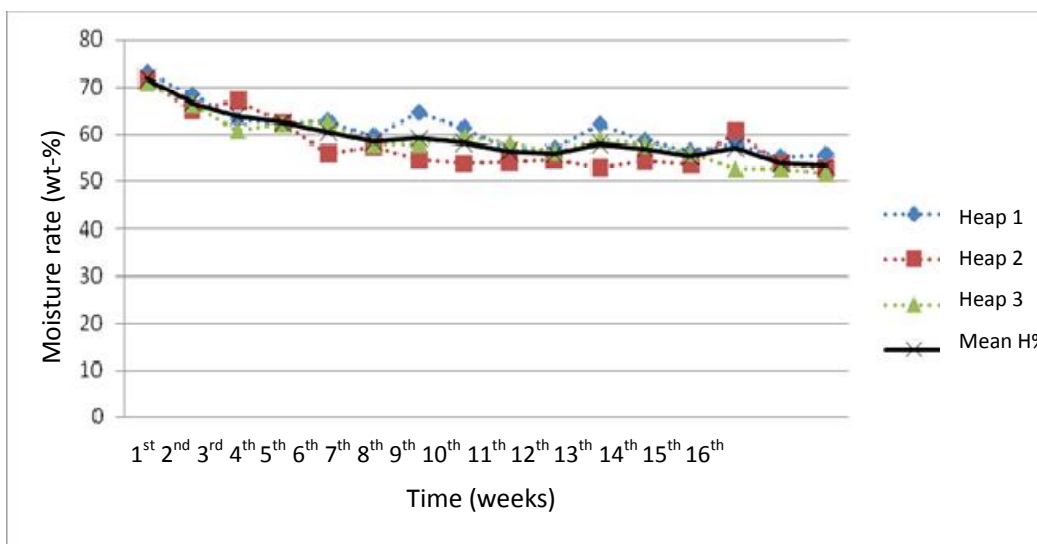


Fig. 3. Evolution of moisture during the process of composting

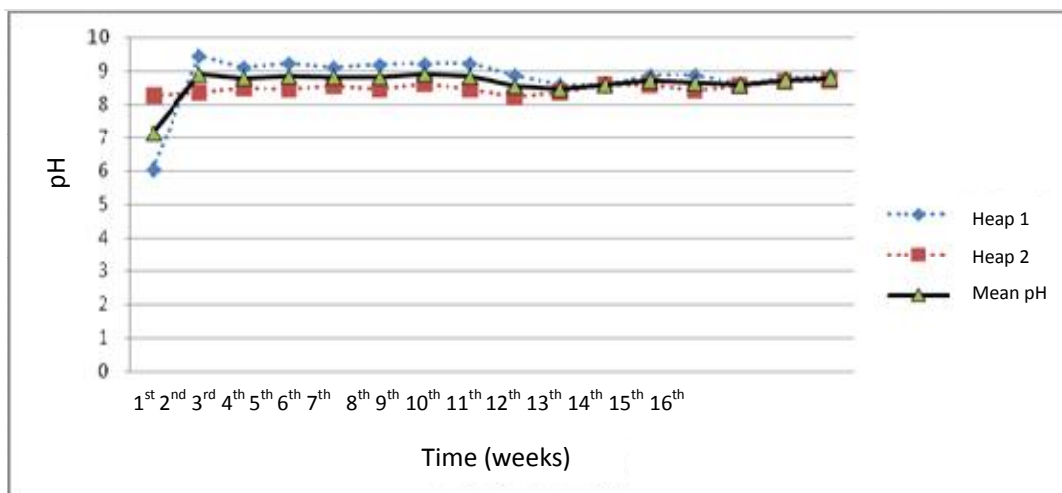


Fig. 4. Evolution of the pH during composting

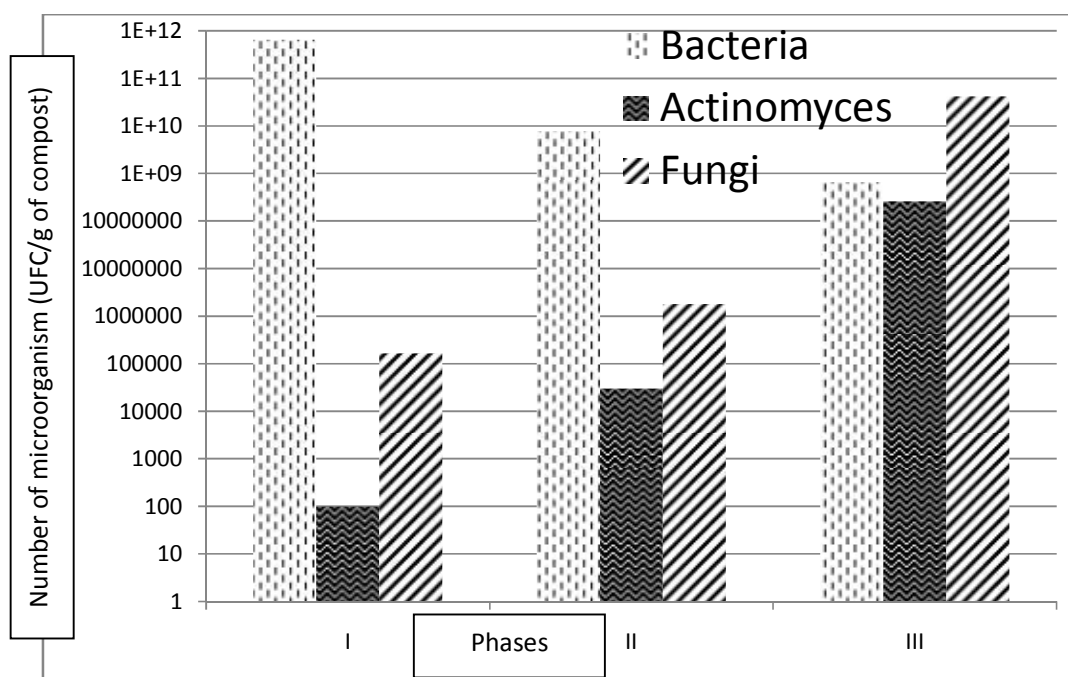


Fig. 5. Evolution of the population of various microbial groups during phases of composting

The bacterial population was 6.5007×10^{11} , 7.80×10^9 and 6.58×10^8 CFU/g compost respectively for the phase 1, phase 2 and phase 3, being a percentage of 98.72, 1.19 and 0.09 percent of the total population (Fig. 5). The decrease in the density of bacteria with the phases of composting observed in this study may be linked to the physico-chemical conditions of the environment and to the availability of nutrients. In fact, according to some authors [26,27], during the early stages of composting, the presence of a large amount of biodegradable organic compounds stimulates microbial growth and enzyme activities. Levanon and Pluda [28] after having isolated bacteria from a mixture of compost from waste of cattle and chickens after 10 weeks of composting equally found that the total bacterial population decreases during the composting process in favor to that of actinomycetes and fungi. This decrease could also be explained by the fact that with the evolution of the process, the amount of degradable organic substances and moisture decreased in the compost. In these conditions, the pH became alkaline and the competition between the microorganisms became more important.

In addition, with the presence of biological regulators in the environment which are responsible for regulating the populations of

other organisms of compost through predation and grazing, the density of bacterial microflora underwent a sharp decline [8,29]. However the increase of the bacterial cell density in the thermophilic phase could be explained by the fact that the bacteria present in the raw material, dominated in quantity and diversity. This could be justified by the fact that the bacteria by their small size had a very high surface/volume ratio, enabling them to have a rapid transfer of soluble substrates to the interior of the cell, which often ensures their predominance over microorganisms of larger dimensions like fungi [7]. One other advantage of the bacteria is the ability, for some of them such as *Bacillus*, to protect itself by producing highly resistant spores against heat, radiation and chemical disinfections [30].

Unlike the bacterial microflora, actinomycetes and fungi increased with the different phases of composting. It was in this way that the population of actinomycetes was of the order of 10^2 , 3×10^4 and 2.6001×10^8 CFU/g compost respectively for the phase 1, 2 and 3, with a percentage of colonization of 0.01 and 99.99 percent for the phase 2 and phase 3. During the thermophilic phase (phase 1), the degradation of the organic matter was more accelerated, characterized by a gradual increase of the temperature and a high production of the heat as a result of high microbial metabolism. The low presence of

actinomycetes can be at the origin of the decrease in the microbial density because they produce antibiotics which inhibit the growth of other microorganisms. The increase of the actinomycetale and fungal community observed during the composting of household waste from the city of Dschang can be explained by the fact that the competition that existed between bacteria, fungi and actinomycetes declined due to the availability in the environment of nutrient reserves for fungi and actinomycetes such as cellulose and lignin. The same trends were observed by [31,32].

3.5 Evolution of Microbial Strains

Table 2 indicates the behavior of different microbial strains during the different phases of composting. The *Pseudomonas* spp which are aerobioses bacteria Gram-negative were present in all the phases. Their number varied between 7.5×10^6 and 6.5×10^{11} CFU/g of compost and constituted more than 98% of the total population of bacteria. They were very important at the beginning of composting and declined until the mature phase (phase 3) of compost. These are nitrogen fixing bacteria, which use very large varieties of organic compounds as a source of carbon and energy. The *Penicillium* spp and *Rhizopus* spp, are microscopic fungi in the form of molds which are very numerous in the mature phase (phase 3), with a similar percentage equal to 99%. The *Mucor* spp strain totally disappeared in the cooling phase (phase 2) of composting and reappeared at the mature stage, when conditions were favorable. The *Nocardia*, strains of actinomycetes (bacteria Gram-positive) developed into fungus. They were present at all the phases but increased significantly from the thermophilic phase (phase 1) where they were lower to phase 3 where the colony increased. The *Saccharomyces* spp, microscopic fungi of the yeast type appeared only in phase 3 of the

compost when it was already matured with a population of 1.91×10^5 CFU/g compost.

3.6 Evolution of Communities

The fungal community was comprised of strains belonging to the group of mushrooms. In this work, among the fungal strains isolated and identified, *Penicillium* spp and *Rhizopus* spp, microscopic fungi in the form of molds were very numerous in the mature phase (phase 3), with a percentage of 99%. The *Mucor* spp strain disappeared in the cooling phase (phase 2) of composting and then appeared in phase 3 (maturation). As for the *Saccharomyces* spp, they appeared only in phase 3. In fact, the fungal strains were highly represented in the mature phase of composting to the disadvantage of the *Pseudomonas* spp which decreased but persisted throughout the process. As has been observed by [20], this change in the fungal structure is probably at the origin of some catalytic abilities of mature compost like the protease activity which has been observed only in the mature composts and these fungal strains observed, are known for their high protein activity. The presence of a morphotype diversity of microorganisms observed in the compost of Dschang could be due to the physico-chemical conditions and the presence of specific nutrients in certain microorganisms that promote their development. The greater diversity observed in our case is especially the abundance of fungal morphotypes at the mature stage. From microbial numbering, [33] have observed that the diversity increased during the period of maturation simultaneously due to a decrease in high microbial activity. The diversity of morphotypes could result from the fact that, during the maturation, the products of the catabolism of recalcitrant compounds were more numerous than in the "young" composts and this could lead to an increase in the number of species.

Table 2. Evolution of the population of the various microbial strains during composting phases

Strains (UFC/g compost)	Phases			Total
	1	2	3	
<i>Pseudomonas</i> spp	$6,5007 \times 10^{+11}$	$7,80 \times 10^{+9}$	$6,58 \times 10^{+8}$	$6,5853 \times 10^{+11}$
<i>Penicillium</i> spp	$4,50 \times 10^{+4}$	$1,60 \times 10^{+6}$	$4,050003 \times 10^{+10}$	$4,0502 \times 10^{+10}$
<i>Rhizopus</i> spp	$1,20 \times 10^{+5}$	$1,90 \times 10^{+5}$	$5,0028 \times 10^{+8}$	$5,0059 \times 10^{+8}$
<i>Mucor</i> spp	$1,5 \times 10^{+3}$	0	$3,0009 \times 10^{+8}$	$3,00092 \times 10^{+8}$
<i>Nocardia</i>	$1 \times 10^{+2}$	$3 \times 10^{+4}$	$2,6001 \times 10^{+8}$	$2,600401 \times 10^{+8}$
<i>Saccharomyces</i> spp	0	0	$1,91 \times 10^{+5}$	$1,91 \times 10^{+5}$

4. CONCLUSION

The objective of this work was to characterize micro-organisms in the compost and to follow the evolution of the microbial communities during the process of composting household waste in the city of Dschang. During the composting, the pH varied between 7.14 and 8.91 with a moisture content of between 55.28 and 70.85%. Three major groups of micro-organisms were present in the studied compost. The density of bacteria decreases with phases of composting while actinomycetes and fungi increased. Among the microbial strains identified, some were presented in all phases (*Pseudomonas*, *Penicillium*, *Rhizopus* and *Nocardia*) others disappear in phase 2 (*Mucor* spp) and others finally appeared only at the stage of maturation (saccharomycetes). The presence or absence of certain groups or strains depended on certain parameters such as the modification of the structure of the fungal environment, the physico-chemical conditions and availability in the medium of nutrient reserves specific to certain groups of microorganisms that promoted their development.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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