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Physical and Chemical Properties of Animals ' Organic Residues Decomposed by Musca domestica and Calliphora vomitoria Larvae

Bloukounon-Goubalan A.Y.[1], Saïdou A.[2],TogbéE.[3], Chabi F.[4], Babatoundé S.[5],Chrysostome C.A.A.M.[6],Kenis M.[7], Mensah G. A.[8]

Abstract

Our study aims to assess physical and chemical properties of organic residues decomposed by fly larvae to be promoted as organic manure. Three stages of four days duration each of biodegradation of 12 various animal substrates (pig, poultry, guinea fowl, sheep, cow, mixture of pig and sheep substrates, mixture of pig and cow substrates, mixture of poultry and sheep substrates, mixture of poultry and cow substrates, mixture of guinea fowl and sheep substrates, mixture of guinea fowl and cow substrates) using *Muscadomestica* and *Calliphoravomitoria* larvae were performed. Temperature, pH, weight, organic carbon, total nitrogen, nitrite and ammonia contents during decomposition process were data collected. The highest temperatures were recorded respectively in the pig and poultry substrates. The pH changed from neutral to alkaline. Organic carbon content reduced during the biodegradation process and was positively correlated with reduction of substrate's weight. Significant decrease of ammonia content was recorded respectively during the first, second and third stages of biodegradation process in the poultry substrate (80, 46 and 61%), guinea fowl (77, 26 and 27%) and pig substrate (76, 66, and 40%). Furthermore, nitrite content was low ranging from 0.0033 to 0.075 gkg^{-1}. The stability and maturity of the final product were high with pig substrates, mixture of poultry and sheep substrates, mixture of poultry and cow substrates, mixture of pig and sheep substrates. It is suggested to give priority to the later substrates for soil fertility management.

Key Words: Soil fertility, manure, biodegradation, organic residues quality, Benin.

Introduction

Erosion, nutrient deficiency, low organic matter level, aluminum and iron toxicity, acidity, crusting, and moisture stress are constraints facing African soils (Place et al., 2003).

[1]Research Unit onIntegrated Soil and Crops Management (ISCM), Laboratory of Soil Sciences, Faculty of Agronomic Sciences, University of Abomey-Calavi, 03 BP 2819 RP Cotonou, Benin.
[2]Research Unit onIntegrated Soil and Crops Management (ISCM), Laboratory of Soil Sciences, Faculty of Agronomic Sciences, University of Abomey-Calavi, 03 BP 2819 RP Cotonou, Benin.
[3]Laboratory of Crop Biology, Faculty of Agronomics Sciences, University of Abomey-Calavi,01 BP 526 RP Cotonou, Benin.
[4]Research Unit onIntegrated Soil and Crops Management (ISCM), Laboratory of Soil Sciences, Faculty of Agronomic Sciences, University of Abomey-Calavi, 03 BP 2819 RP Cotonou, Benin.
[5]Laboratory of Aviculture and Zoo-economy research, Faculty of Agronomic Sciences, University of Abomey-Calavi,03 BP 2819 RP Cotonou, Benin.
[6]Laboratory of Crop Biology, Faculty of Agronomics Sciences, University of Abomey-Calavi,01 BP 526 RP Cotonou, Benin.
[7] CABI, Rue des Grillons 1, 2800 Delémont, Switzerland
[8] Laboratory ofZootechny, Veterinary and Fishery research, CRAAgonkanmey, National Institute of Agriculture Researches (INRAB), 01 BP 884 RP, Cotonou, Benin.

These constraints, result from the declining of soil organic matter (Barnardanddu Preez, 2004) which is now recognized by soil scientists as a major factor controlling the capacity of soil resources (Manlayet al., 2007). The problem is alarming in Benin with the ferralitic soilin the south on which current researches reported very low organic matter content (Bloukounonet al., 2015; Iguéet al., 2013; Balogounet al., 2013; Igué, 2009; Saïdouet al., 2003).Bloukounonet al. (2015) reported a low quality of organic matter and the immobilization of nitrogen in the Acrisol of the Adja plateau (southern Benin). Even, Igué (2009) point out the declining of soil organic matter (1.0% after 10 years under continuous cultivation and 0.6% after 25 years)in southern Benin. These soil types present risk of calcium and magnesium lixiviation due to solubilization of Aluminum caused by soil organic matter decline. The consequences are oxidation and leaching of ammonium involving soil acidification and modification of nitrogen cycle. Therefore, restoration of soil organic matter using high quality of organic manure seems to be solution since the natural fallow cannot be adopted in the region due high population density. Several type of organic material such as animal manures, crop residues and organic wastes are available and already used as organic manure. But, most of them are sometime immature and poorly stabilized and may also be source of nitrogen immobilization in the soil. In fact, the immature residues could create anaerobic conditions and phytotoxicity of ammonia gas and release some organic acids (Huang et al., 2004). They may also lead to immobilization of plants nutrients and cause soil and water pollution (Cambardellaet al., 2003). Thus, decomposition process of the organic material before using as manureis necessary for sustainable management of soil fertility. Composting and vermicomposting have been developed but poorlyadopted by farmers in Benindue to their constraints and the high production cost. On the other side, the useof the potential of the insects' larvae to decompose the organic was tecould be an opportunity for smallholder farmer with limited resources. In fact, many insects naturally feed on organic wastes, incorporating the nutrients into their bodies and reducing the amount of waste material (Čičkováet al., 2015). With the dipteran larvae, the bioconversion of waste may result in significant production of fly biomass and digested manure, which can be source of revenue for farmers (Wang et al., 2013).Also, the mass of emerging larvae can also be used as source of protein for poultry in substitution to fish and the final material as organic manure. The biodegradation of the organic waste bythe dipteran larvae is fast due to the short development cycle of the insect. The process may take 4–30 days, depending to the species (Myers et al., 2008).It was demonstrated that, the black soldier fly larvae (*Hermetiaillucens*) degrade easily organic material of different origins such as domestic waste, poultry, pig and cow manure and even human excreta (Dieneret al., 2011; Banks et al., 2014).

Waste reduction by these fly larvae is beyond 50% (Sheppard et al., 1994) depending on the daily amount of waste added to the experimental unit and presence/absence of a drainage system (Dieneret al., 2011). Čičkováet al. (2012) demonstrated that *Muscadomestica*, the most frequently found species reduce 1 kg of pig dejectionto 0.18 kg. Zhang et al. (2012), Zhu et al. (2012), Zhu et al. (2015) and Wang et al. (2016) reported also an important loss of carbon and nitrogen in the swine manure after housefly (*Muscadomestica*) larvae production. Such potential of housefly can be harnessed in rural areas under the tropical conditions, especially in Sub-Saharan Africa. There is thus a need to elucidate those results for further local adaptation in a context of agriculture intensification. This paper aims to assess the ability of the fly larvae in the improvement of the physical and chemical properties of the organic manures produce from the biodegradation process of the organic residues and their contribution to the maturity and stability of different types of substrate. Specifically, it aims i) to assess the physical properties (pH, temperature and substrate weight) of the organic residues during the biodegradation process by the fly larvae; ii) to evaluate nutrients (carbon, total nitrogen, N-$NO_3^-$and N-NH_4^+) contents in the manures produce after the biodegradation process; iii) to make a typology of these organic substrates regarding their physical and chemical properties and their fly larvae's production in the perspective of production of stable and mature bio-fertilizer for soil fertility restoration.

Material And Methods

Biodegradation process and experimental design

The study was carried out from March to June 2016 at the experimental station of the Faculty of Agronomic Sciences of University of Abomey-Calavi in Benin. Single or mixed animal manures were used: pig substrate, poultry substrate, guinea fowl substrate, sheep substrate, cow substrate, mixture of poultry and sheep substrates, mixture of poultry and cow substrates, mixture of pig and sheep substrates, mixture of pig and cow substrates, mixture of guinea fowl and sheep substrates, mixture of guinea fowl and cow substrates, leading to 11 treatments. 1:1 ratio was used for the mixed substrates.

All of these substrates underwent 12 days of biodegradation process subdivided into three stages using housefly *Muscadomestica* and bluebottle fly *Calliphoravomitoria*. Each stage corresponded of four days duration which ended up with the harvest of the fly larvae. The biodegradation process occurred in the plastic containers of 15 cm of depth and 50cm diameter. Three kilograms of each organic substrate, with 65 to 75% moisture content, were put in the plastic container and exposed in ventilated shed for natural fly ovipositor. Ambient temperature during the experiment ranged between 25 and 37°C. Ten hours after natural ovipositor, the plastic containers were covered with a net in order to obtain larva cohort of the same generation. After harvesting the larvae, 1 kg of new fresh organic substrate was added to the remaining organic substrate for the second stage of biodegradation. The same was applied to the remaining substrate for the third stage biodegradation. Completely randomized block design with seven replications was setting up. The treatments consisted of the 11 single or mixed organic substrates mentioned previously. Manures were collected from various farms in the district of Abomey-Calavi (southern Benin), two or three days after the release by the animals. The substrates were collected from the exotic breed of pig and poultry also from the local breedofguinea fowl, sheep and cow.

Sampling and data collection

Each organic substrate was sampled at the beginning and at the end of each biodegradation stage. These samples were conserved at -4°C for laboratory analysis in order to stop microbial activities. Physical parameters measured concerned, temperature and weight of the substrates at the end of each biodegradation stage. The pH, temperature and the weight of the substrates were measured daily during the 12 days of biodegradation process. At the beginning and the end of each biodegradation stage. The chemical parameters measured concerned organic carbon, total nitrogen, $N\text{-}NH_4^+$ and $N\text{-}NO_3^-$ content. The C:N and $NH_4^+:NO_3^-$ ratios were calculated to evaluate the maturity (Bernal et al., 1998) of each substrate after larvae biodegradation. The total quantity of larvae of Musca and Calliphora fly was determined in each substrate using the following formula:

Larva productivity (gkg^{-1}) = (Larva weight (g DM))/(Organic substrate weight (kg DM))

Where DM means dry matter.

Laboratory analysis

The analyses were carried out in the Laboratory of the International Crops Research Institute for the Semi-Arid Tropics (ICRISAT-Niger) at Niamey. The organic carbon was determined firstly after drying in an oven at 105°C and ashing in a furnace at 550 °C for four hours. Total N was determined by wet digestion with a mixture of sulfuric acid (H_2SO_4), salycilic acid, hydrogen peroxide (H_2O_2) and selenium.$N\text{-}NO_3^-$and $N\text{-}NH_4^+$ were determined by weighing 0.5 g of substrate sample then 30ml of 2N KClwere added on the sample following by shaking for 20 minutes. The mixture was filtered through mineral N filter paper, then the extract was stored at -18°C until measurement. Total and mineral N measurement were done by colorimeter with auto-Analyzer using Berth toleration.

Statistical analysis

Statistical analysis were performed using repeated measurement with GLM procedure of the R 3.2.1 software. During the analysis, each organic substrate was considered as factor. The Student Newman-Keuls test was used for mean separation and the significant level was set at 5%.Relation between the physical and chemical properties on the one hand and the larva population on the other hand was established using the principal components analysis then the different types of organic substrate were categorized.

Results

Change of the organic substrates physical properties during the biodegradation process by the fly larvae Temperature and pH

The temperature and pH of the organic substrates during the biodegradation process varied significantly ($P<0.05$) as presented in the Table 1. The temperature rangedbetween29 and39°C (Fig. 1 A). The highest mean temperatures were recorded with the pig substrate, poultry substrate, mixture of poultry and sheep substrates and mixture of poultry and cow substrates.

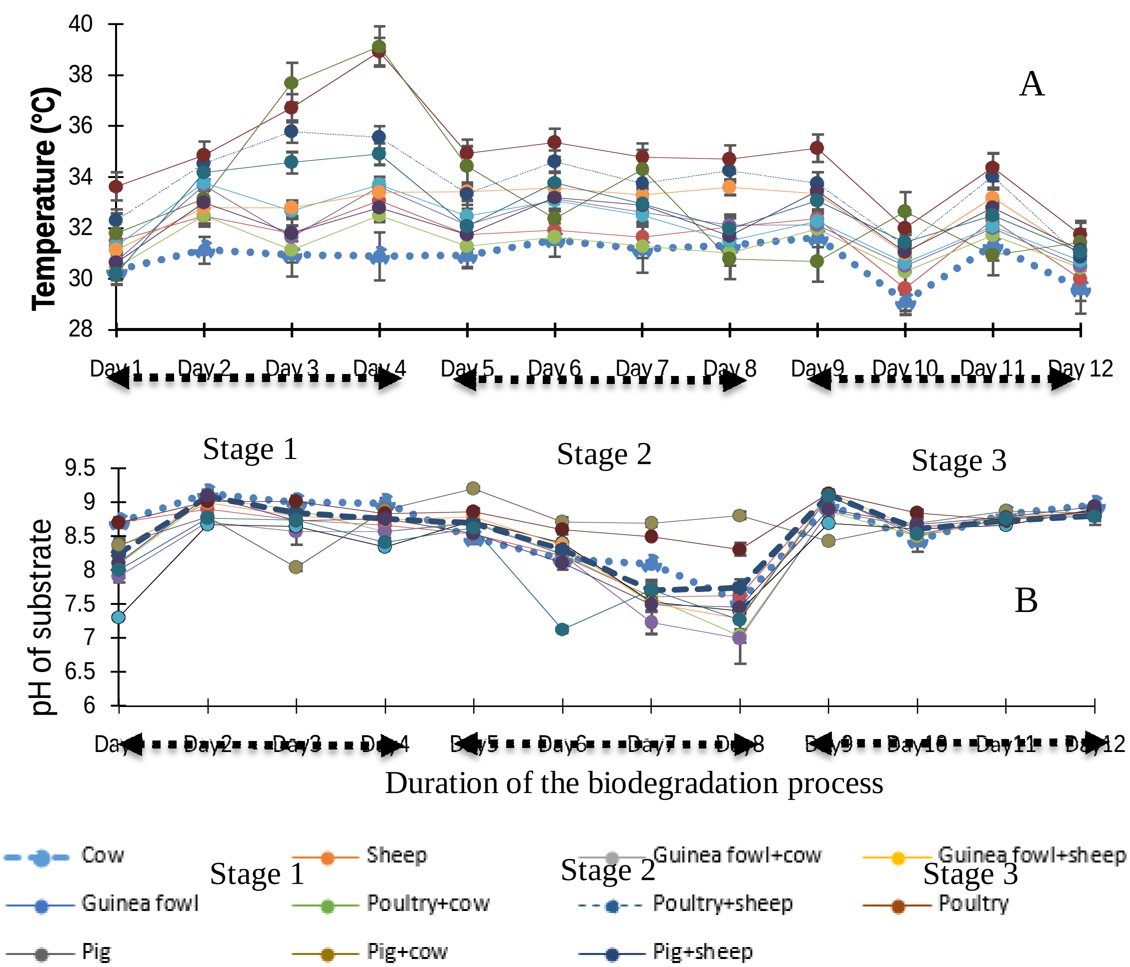
In general, the pig substrate gave the highest temperature. The temperature of the pig substrate increased rapidly from37°C after three days of biodegradation to39°Cafter four days biodegradation and decreased to 31°C after 12 days of biodegradation. However, the temperature of the poultry substrate reached 38°C after three days biodegradation and decreased to 31°C after 12days. The peaks of the temperature were reached after three and four days of biodegradation by the fly larvae. The lowest temperatures during the biodegradation stages were recorded with the cow substrate, sheep substrate and the mixture of the guinea fowl and cow substrates. The pH fluctuated during the larvae biodegradation stages andrangedbetween7 and9as shown in Fig. 1B. After 12 days of biodegradation the value of the pH achieved 8.5.

Table 1: Result of the analysis of variance (F values) of the repeated measurements of the temperature and pH.

Source of variation	Degree of freedom	Temperature	pH
Organic substrates	10	42.81***	13.85***
Duration of biodegradation	11	35.06***	118.50***
Substrate x Duration of biodegradation	110	7.01***	3.94***

*** : P<0.0001

Figure 1: Variation of the temperature (A) and pH (B) during the biodegradation process by Muscaand Calliphora larvae of the different types of organic substrates after 12 days duration



Variation of the organic substrates weight during the duration of the biodegradation process

The organic substrates weight declined significantly ($P < 0.05$) from the beginning to the end of each stage of biodegradation process (Fig. 2).

During the first stage of biodegradation, high weight loss(26, 20 and 19%)were recorded respectively with the mixture of poultry y and cow substrates, mixture of poultry and sheep substrates and pig substrate alone. While low weight loss 3, 6 and 7% were recorded respectively with sheep substrate alone, mixture of pig and sheep substrates, mixture of pig and cow substrates. During the second stage of the biodegradation process by the fly larvae, the mixture of pig and sheep substrates, pig substrate alone, admixture of poultry and cow substrates registered higher weight loss compared with the remain substrates respectively 26, 22 and 17%. Whereas, the highest weight loss (20%) during the third stage of biodegradation was recorded on poultry substrate alone. In general, considering the whole biodegradation process, mixture of poultry and cow substrate, mixture of pig sheep substrate, mixture of pig and cow substrate, pig substrate, poultry substrate and cows substrate registered significant ($P < 0.001$) weight losses.

Evolution of the organic carbon, total nitrogen, N-NO3- and N-NH4+ content during the biodegradation process of the substrates by the fly larvae

Significant differences ($P < 0.05$) were founding the organic carbon, total nitrogen, $N\text{-}NO_3^-$ and $N\text{-}NH_4^+$ content during the biodegradation process of the substrates. At each biodegradation stage, similar trends were found(Fig. 3). At the beginning of the biodegradation process, the organic carbon content varied between195.0±13.0g kg^{-1} (poultry substrate) and342.5±20.0 g kg^{-1}(cow substrate). However, at the end of the experiment it varied between115.0±4.5g kg^{-1} (mixture of pig and sheep substrates) and339.5±19.5 g kg^{-1} (cow substrate). But, during the first stage of the biodegradation process, a decrease of the organic carbon content of 39 and 35% were recorded respectively in the cow and sheep substrates. During the second stage, the highest decrease (25%) of the organic carbon content was recorded in the mixture of pig and cow substrates and the lowest (19%) in the mixture of pig and sheep substrates. During the third stage of biodegradation, a significant ($P < 0.05$) decrease (55%) of the organic carbon content was noticed in the mixture of pig and sheep substrates, 30%in the pig substrate and25%in the mixture of poultry and cow substrates. Considering the 12 days duration of the biodegradation process, an important decrease of the organic carbon contents were recorded in the mixture of pig and sheep substrates (219 gkg^{-1}), cow substrate (157.5 gkg^{-1}), pig substrate (153.5 gkg^{-1}), guinea fowl substrate (131.5 gkg^{-1}), mixture of poultry and cow substrates(114.5 gkg^{-1})and mixture of poultry and sheep substrates (110.5 gkg^{-1}) as presented in Fig. 3A.

The total nitrogen content revealed a significant ($P < 0.05$) decrease in the poultry and pig substrates. During the first stage of biodegradation it was 44%and 45% respectively in the poultry and the pig substrates. Considering all of the stages of the biodegradation process, the poultry and pig substrates, mixture of pig and cow substrates, mixture of poultry and cow substrates, mixture of pig and sheep substrates and then mixture of poultry and sheep substrates registered significant ($P < 0.05$) decrease of the total nitrogen content (Fig. 3B). The$N\text{-}NH_4^+$content decreased significantly ($P < 0.05$) as the total nitrogen content in the poultry substrate, guinea fowl substrate, pig substrate, mixture of poultry and cow substrate sand in the mixture of poultry and sheep substrate(Fig. 3C). During the first stage of the biodegradation process, a decrease of 80, 77, 76 and 74% of the $N\text{-}NH_4^+$contentswere registered respectively in the poultry substrate, guinea fowl substrate, pig substrate and in the mixture of poultry and cow substrates. During the second stage, an important decrease of 76 and 67% of the $N\text{-}NH_4^+$contents were noticed respectively in the mixture of guinea fowl and sheep substrates and in the pig substrate alone. Whereas, during the third stage, a decrease of 71and 61% of the $N\text{-}NH_4^+$contentswere registered respectively in the mixture of poultry and sheep substrates and in the poultry substrate alone. In general, the $N\text{-}NH_4^+$ contents at the beginning of the biodegradation (day1) ranged between 0.2 gkg^{-1} (for cow substrate) and 4.35 gkg^{-1}(for poultry substrate) whereas, at the end (day12) it ranged between0.15gkg^{-1} (for cow substrate) and 0.7gkg^{-1} (for the mixture of pig and cow substrates).The $N\text{-}NO_3^-$contents in all of the substrates were low during the biodegradation process. It varied between 0.0033 and0.075 gkg^{-1} (Fig. 3D). In opposite with the trend observed with the other nutrients, the $N\text{-}NO_3^-$content in the poultry substrate, mixture of poultry and sheep substrates and mixture of pig and cow substrate sat the end of experiment, have increased compared with the initial substrates.

Figure 2: Substrate weight reduction during biodegradation by fly larvae

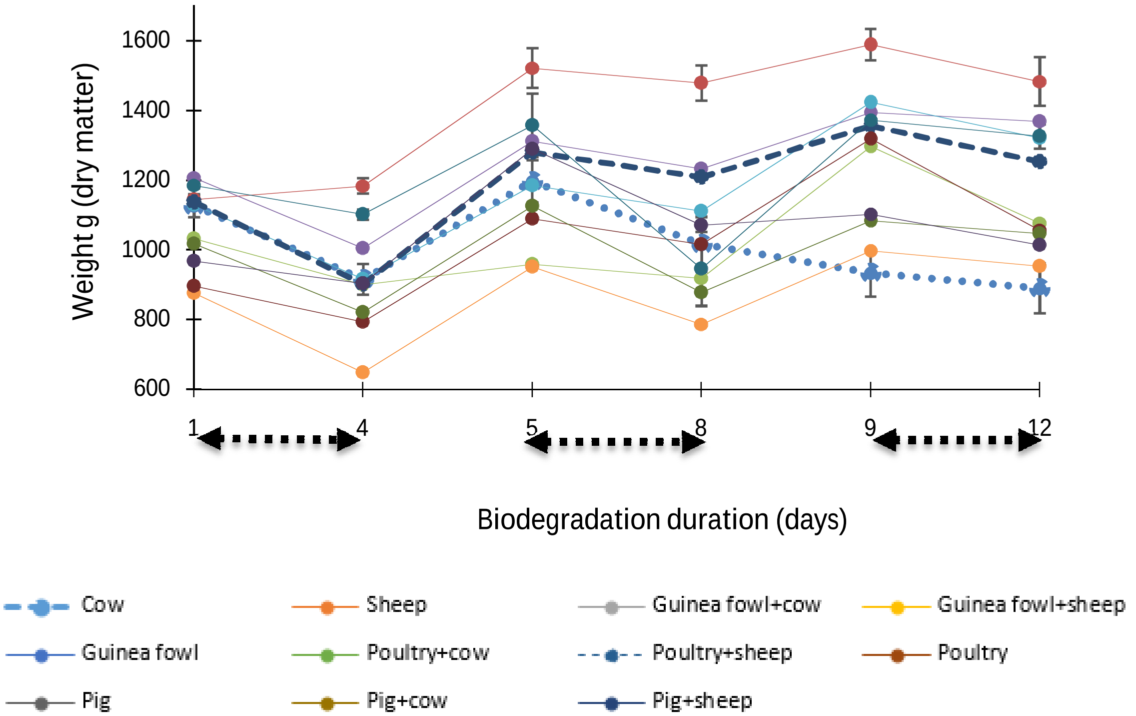


Fig. 4 presents the change of the C:N and $N\text{-}NH_4^+$:$N\text{-}NO_3^-$ ratios in the different single and mixture of substrates during the biodegradation process by the fly larvae. A part from the cow substrate, guinea fowl substrate, mixture of guinea fowl and cow substrates, mixture of pig and cow substrates; the C:N ratios were below10 at the end of the experiment. High decrease of the C:N ratio (from 15 to 8) was observed in the mixture of poultry and cow substrates. At the end of the first stage of the biodegradation process, only the single poultry and sheep substrates presenteda C:N ratio below10. The poultry substrate, pig substrate, sheep substrate, mixture of poultry and cow substrates, mixture of poultry and sheep substrates and mixture of pig and sheep substrates showed the lowest C:N ratios (between7 and9) at the end of the second and third stages of the biodegradation process. The lowest C:N ratio was recorded with the single poultry substrate at the beginning and at the end of the experiment(Fig. 4A). The cow substrate showed the highest C:N ratio, 23 and 21 respectively at the beginning and the end of the experiment. Furthermore, the $N\text{-}NH_4^+$:$N\text{-}NO_3^-$ ratios decreased significantly ($P < 0.05$) during the biodegradation process (Fig. 4B). It decreased from 94 to 13 in the single poultry substrate, from 72 to 15 in the guinea fowl substrate,63 to 15 in the mixture of poultry and cow substrates, from 60 to 10 in the mixture of poultry and sheep substrates and from 48 to 18 in the single pig substrate. These decreases were observed at the first stage of biodegradation where the $N\text{-}NH_4^+$:$N\text{-}NO_3^-$ratios were below 31. The single cow and sheep substrates showed the lowest $N\text{-}NH_4^+$:$N\text{-}NO_3^-$ ratios during the experiment with values varying respectivelybetween5 and6; and between 27 and8.

Figure 3: Variation of the organic carbon (A), total nitrogen (B), N-NH_4^+(C) and N-NO_3^-(D) contents in the single and mixture of substrates during the biodegradation process by the fly larvae.

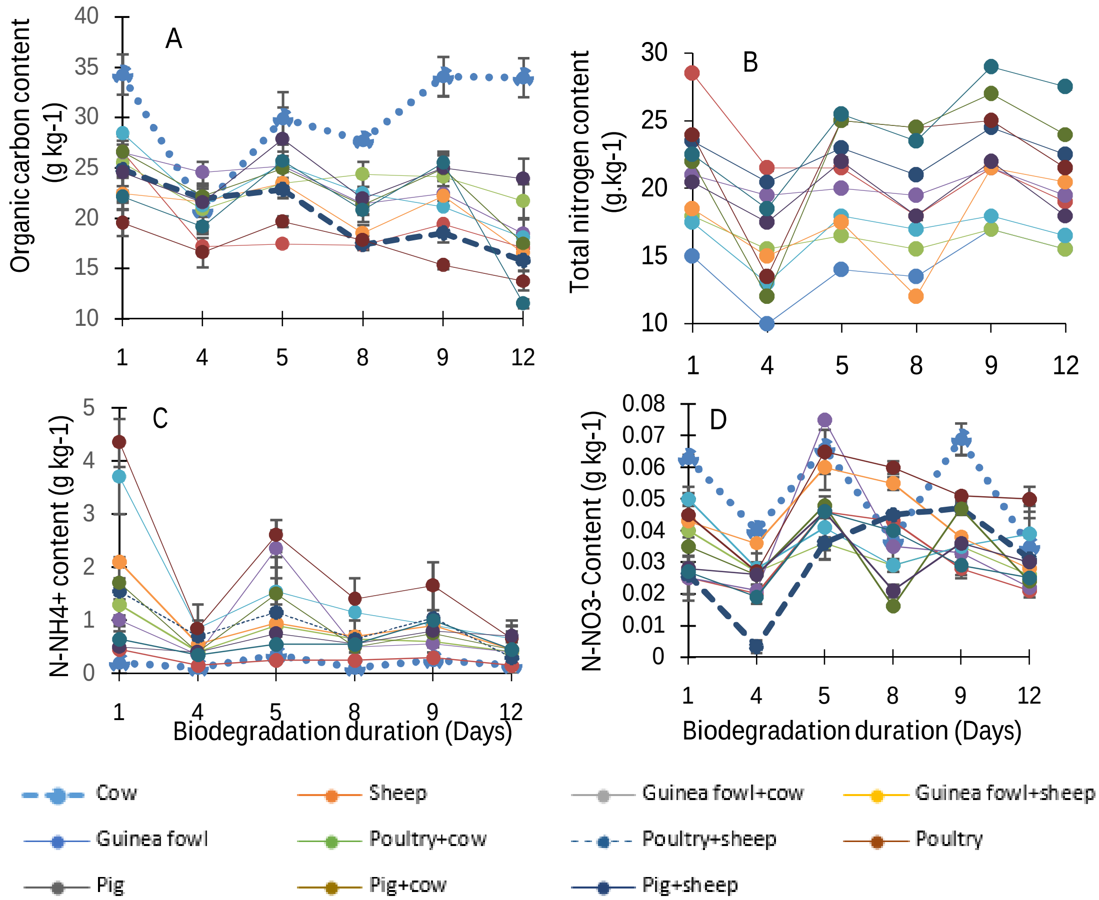
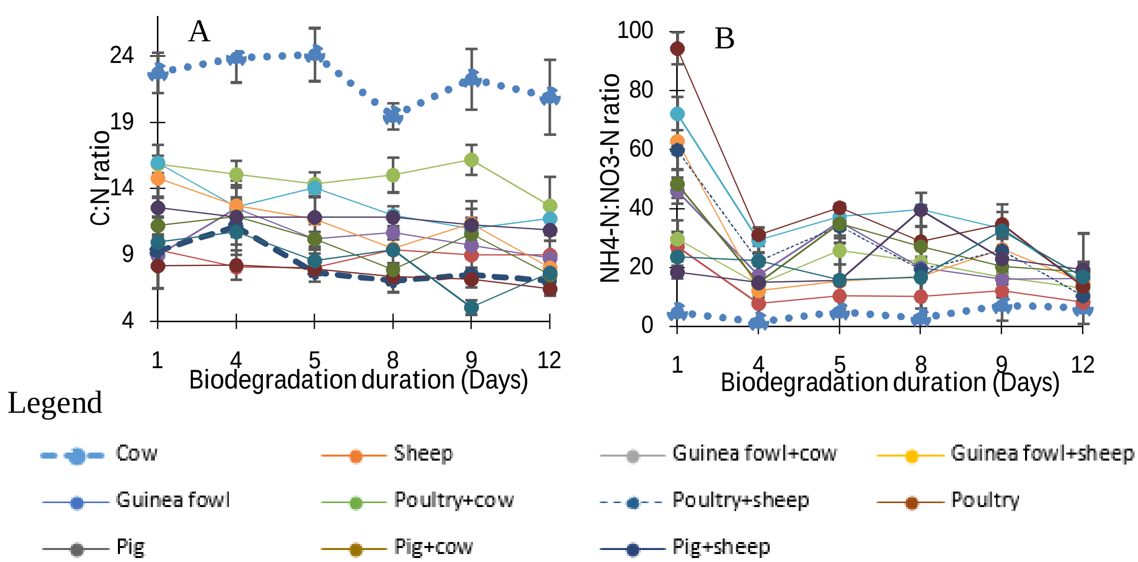


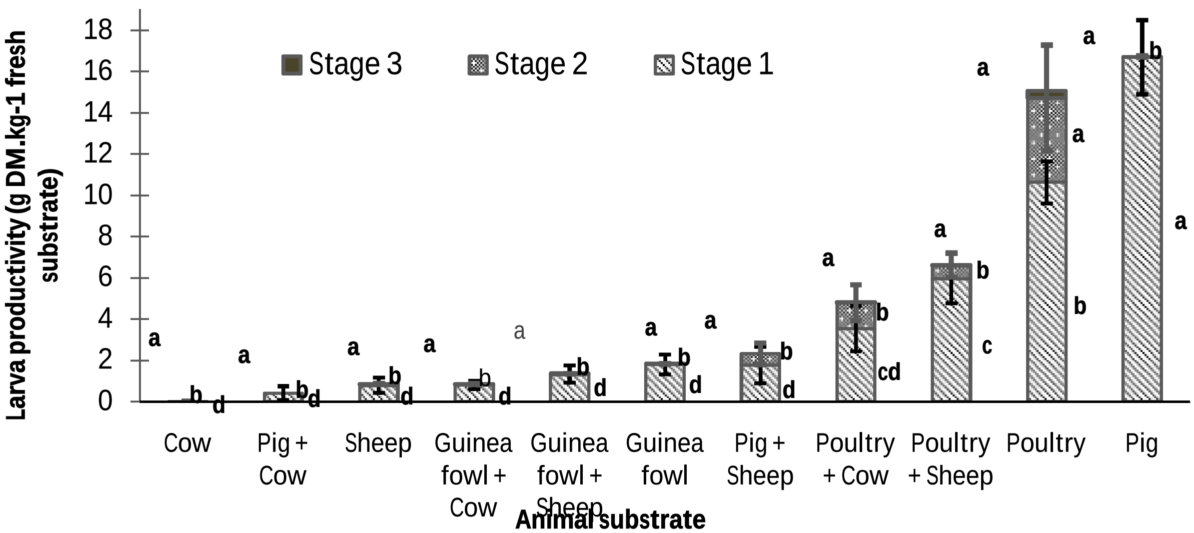
Figure 4: Variation of the C:N (A) and N-NH_4^+:N-NO_3^- (B) ratios in the single and the mixture of substrates during the biodegradation process by the fly larvae



Typology of the substrates degraded by the fly larvae

Potential of each type of single and mixture of substrates to produce larvae during the biodegradation process Fig. 5 presents the potential of each type of substrate to produce larva during the different stages of the biodegradation process. The fly larvae production was important with the single pig substrate followed by that of poultry, mixture of poultry and sheep substrates, and mixture of poultry and cow substrates. The total mass of larva produced with the single pig substrates were respectively 1.1, 2.5 and 3.5 times higher than that of poultry substrate, mixture of poultry and sheep substrates and mixture of poultry and cow substrates. The cow substrates produced the lowest larva mass (0,008 g DMkg^{-1}) considering all of the stages of biodegradation. During the first stage of biodegradation, the weight of larvae produced with pig's substrate was significantly ($P<0.05$) higher than that of poultry. Also, at the same stage, the weights of larvae produced by poultry substrate were significantly higher than those produced on the mixture of poultry and sheep substrates and mixture of poultry and cow substrates. During the second stage, the weight of the larvae produced with the single pig substrate was low while, poultry substrate, mixture of poultry and sheep substrates, mixture of poultry and cow substrates and mixture of pig and sheep substrates produced high amount of fly larvae. During the third stage, the weight of the fly larvae produced with each substrate was almost nil.

Figure 5: **Potential of the different types of single and mixture of substrates to produce Musca and calliphora larvae during the biodegradation process.**

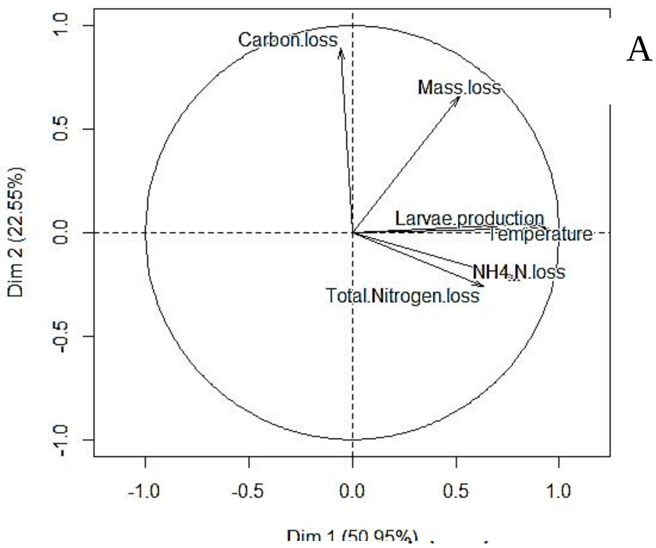


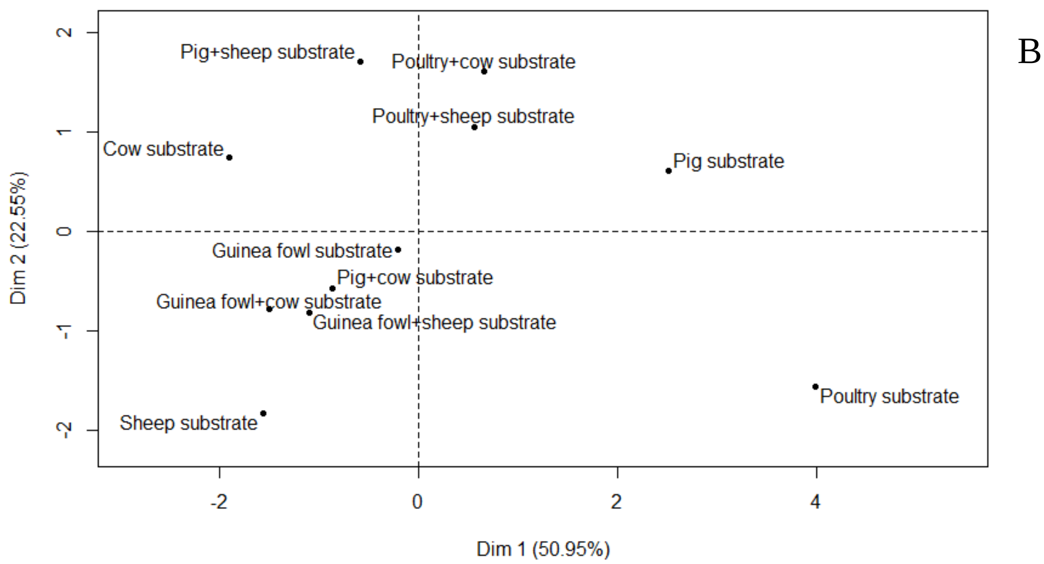
Physical and chemical parameters determining the potential of the substrates to produce fly larvae

The physical and chemical parameters determined in the substrate and the total amount of fly larvae produced regarding the single and the mixture of the substrates were subjected to principal component analysis (Fig. 6).It appear from the result that, 73.50% of the substrate characteristics and larva production were explained by the first two axes. Therefore, only theses axes were used to describe the relationship between the chemical and physical characteristics of the substrates during the biodegradation process and the amount of larvae produced. Correlation between variables revealed that, the temperature increase as a result of the amount of larva produced, which was followed by a reduction of total nitrogen, ammonia and substrate mass. Moreover, the substrate mass loss involved a reduction of organic carbon (Fig. 6A). Thus, the projection of substrates in the system of axes (Fig. 6B) indicated that, the biodegradation process of pig substrate by the fly larvae was characterized by an increase of the temperature during the process with a reduction of organic carbon, total nitrogen, ammonia and mass of the substrate. Furthermore, the biodegradation process of the single poultry substrate by the fly larvae was characterized by an increase of the temperature during the process with a reduction of total nitrogen, ammonia and substrate's mass.

However, the biodegradation process of the mixture of pig and sheep substrates, mixture of poultry and cow substrates and mixture of poultry and sheep substrates were characterized by a reduction of organic carbon content and substrate's mass. Cow substrate, sheep substrate and the mixture of guinea fowl and sheep substrates were poorly degraded by the fly larvae which result edin lower temperature, and a few reduction of total nitrogen content, ammonia content and the mass of the substrate.

Figure 6: **Correlation between the substrate characteristics and the fly larvae production (A) and typology of the substrates regarding larvae production (B)**





Discussion

Change in the substrate characteristic during the biodegradation process

The temperature and pH were important indicators of the efficiency of the biodegradation process. A significant increase in the temperature and pH were observed during the biodegradation process. It was noticed in the substrates where an important population of the larvae was observed (poultry, pig, mixture of poultry and sheep substrates, mixture of poultry and cow substrates, mixture of pig and sheep substrates). This indicated that fly larvae have the capacity to alter the medium by rendering it appropriate for their growth.

Those changes were mainly due to the crawling and the digestion by the fly larvae which increased the pore of the material and also improved the metabolism of the micro-organisms in substrate (Zhu et al., 2015). The temperatures recorded during the process were below40°C indicating that the biodegradation of the substrate from animal residues by Musca and Calliphoralarvae in free ovipositor system is occurring in mesospheric phase. Wang et al. (2016) found that, with the aid of housefly larvae, pig substrate reached a hemophilic phase after five days with apeakof 47°C and then decreased to 42°C after six days. In the present study, we could not keep the biodegradation more than four days because larvae of Muscat and Calliphora complete their development in four days under tropical condition (Sanchez-Arroyo and Capinera, 2008). The pH changed from neutral to alkaline (around 8.7). Zhu et al. (2015); Zhu et al. (2012) found the same result during the biodegradation with the housefly larvae.

This change of the pH was due to the production of ammonia during ammonification and mineralization of organic nitrogen as a result of the fly larvae activities. In fact, ammonification reaction produces hydroxyl ions (OH^-), ammonia and increases pH value. Thus, to accomplish their metabolism, fly larvae breakdown the organic residues resulting to the carbon reduction (as CO_2 form), nitrogen decrease (as $N\text{-}NH_4^+$ and $N\text{-}NO_3^-$), and the substrate's weight reduction. Several studies reported these changes during the substrate degradation by the fly larvae compared to the underrated substrate (Barnard et al., 1998; Zhu et al., 2012; Zhu et al., 2015; Wang et al., 2016). Cow and sheep substrates registered the highest organic carbon decrease (around 35%) during the first stage of biodegradation process. The highest reduction of organic carbon observed in the cow and sheep substrates at this stage proved high microbial activity. At this same stage, the substrates with the highest larvae weight (pig, poultry, mixture of poultry and sheep, mixture of poultry and cow, mixture of pig and sheep substrates) showed low organic carbon decrease(around 8%). Zhu et al. (2012) and Zhu et al. (2015) also recorded about 8% decrease of carbon in the pig substrate respectively after seven and six days of biodegradation by the housefly larvae. They justified this result by the low degradation of carbon macromolecular chains by the fly larvae before to reach its cycle. Furthermore, the second and third biodegradation processes were characterized by the highest organic carbon decrease(55 to 21%) in the mixture of poultry and cow substrates, mixture of poultry and sheep substrates, mixture of pig and cow substrates and mixture of pig and sheep substrates. Whereas the fly larvae population was low in the substrate at these stages compared to the first. That result points out an interrelation between fly larvae and microorganisms presents in the substrates. Thus, the different changes observed during the biodegradation of the organic residues by larvae cannot be attributed to the larvae alone which in the process are agents of decomposition and catalyzer of microbial biodegradation at the same time. According to Čičkováet al. (2015), the main contribution of the fly larvae to biodegradation seems to be mechanical aeration which results in increased loss of water, ammonia and favoring the growth of aerobic microorganisms. Sufficient oxygen creates by adequate aeration, stimulates micro-organisms to convert organic compounds into inorganic. So, fly larvae likely benefit from complex metabolic interactions within a diverse bacterial community in a natural environment leading to a rapid degradation of organic material (Zureket al., 2000). This process makes available carbon, nutrients and bacteria as larva food. Thus, in manure bioconversion by fly larva, mesospheric bacteria likely contribute in the interaction with the larva to the material decomposition. This is an evidence of the advantage to continue the biodegradation by adding substrates even if larvae (Musca and calliphora) have completed their cycle.

Contrary to the other substrates, low nitrogen concentration in the cow and sheep substrates did not favorite multiplication of bacteria and was probably the root cause of the low biodegradation of those substrate during the second stage. The total nitrogen decrease was high (45%) with the single pig and poultry substrates. This nitrogen decrease corroborated result of Zhu et al. (2012) with housefly (32%), Newton et al. (2005) with Black soldier (55%) and Myers et al. (2008) with Black soldier (30-50%). It was due to ammonia lost during the process reflected the degree of the breakdown of proteins molecular resulted by larvae activity. The reduction in NO_3^- concentration in the substrates was always lower than that of NH_4^+concentration. That results proves that, larvae use preferentially nitrogen in ammonia form. Then ammonia was not changed in nitrite by nitrification reaction despite the favorable nitrification conditions in the substrate resulting in the low level of NO_3^- concentration during the biodegradation.

Stability and maturity of the product biodegraded by the fly larvae for soil amendment

The stability and maturity concepts are key factors to determine whether biodegraded product is suitable for soil amendment. Maturity is loosely defined as material suitability for plant growth and has often been associated with degree of humification or its phytotoxicity, whereas stability is associated with microbial activity (Bernal et al., 2009). Material including compost and animal manure are ready to be used as organic manure when the C:N ratio < 10; N-NH_4^+ concentration < 500 mg kg^{-1}; N-NH_4^+:N-NO_3^- ratio < 0,16; alkali-extractable organic-C ≤ 60 gkg^{-1} (Bernal et al., 2009; Zmora-Nahum et al., 2005; Bernal et al., 1998). Significant mass reduction, CO_2 reduction rate <4mgg^{-1}organic matterday^{-1} are indicators used to appreciate the stability of organic residues.

At the end of each biodegradation stage, the C:N ratio of the single poultry substrate was <10whileN-NH_4^+concentration was>500 gkg^{-1}indicating that, the material was still immature(CCQC, 2001). The low carbon decrease (around 8%) with the low mass loss registered during the biodegradation process indicated that it was unstable. According to Wang et al. (2004), residues rich in nitrogen, but with low lignin and polyphone content decomposed rapidly and released a large amount of N during the early stages of biodegradation, but may not contribute much to the maintenance of soil organic matter. Thus, poultry substrate biodegraded by the fly larvae regarding experimental condition requires further treatment such as composting before be used as soil amendment. However, single pig substrate was already stable and matures at the end of the second or third stage of biodegradation given C: n Nratio <10, N-NH_4^+ concentration<500 and the significant mass and carbon reduction (around 19%). Wang et al. (2016) found similar results with the pig substrates after having noticed fast biodegradation of dissolved organic matter and a higher level of aromaticity and mummification after six days of biodegradation by housefly larvae. But, a subsequent weight loss will be necessary for more stability by leaving the product without another turnover.n The mixture of pig and sheep substrates, mixture of poultry and sheep substrates and mixture of poultry and cow substrates reached their maturity and stability at the end of the three stages of biodegradation resulting in a substantial weight and carbon loss, as well as a suitable C:N ratios andN-NH_4^+ concentration. However, the maturity and stability of those substrates must be confirmed with mineralization test in the soil and plants growth bioassays.

Contribution of the organic residues biodegraded by the fly larvae to soil nutrients replenishment

In Southern Benin, the most deficient nutrients in the soil are nitrogen and phosphorus (Iguéet al., 2013). Considering the low level of organic matter (Bloukounonet al., 2015; Iguéet al., 2013; Balogounet al., 2013) resulting to the low microbial activity of the soil of the region, N from inorganic fertilizers may not be used by the crops and subjected to leaching and gentrification. Nutrients replenishment in these soils, required organic manure addition to increase soil microorganisms' growth. In the short to medium term, increased soil N supply will depend on regular inputs of organic N sources. Therefore, appropriate strategies consisted to provide sufficient levels of N inputs while at the same time slowly rebuilding stocks (Sanchez, 1997). Thus, single pig substrate, mixture of poultry and sheep substrates, mixture of pig and sheep substrates were more suitable since they presented at the same time the highest total N and C contents at the end of the biodegradation process. Compared with the substrate not decomposed by larvae, the risk of gentrification and leaching in the soil is very low since low N-NO_3^- content at the end of the biodegradation was observed. In short term, these substrates are suitable for the production of short cycle crops' and in medium term they can assure the replenishment of the degraded soil. The low C:Nratio noticed in the substrates could reduce the risk of N immobilization in the soil except the single cow substrate which showed very low N content with C:N ratio >21. In general, mineralization studies of the substrates will more elucidate about their potential in nutrients replenishment.

Conclusion

The three stages of the biodegradation process by *Muscadomestica* and *Calliphoravomitoria* larvae were efficient for the production of organic manure. During this biodegradation process, the temperature of the substrates rises and pH changes from neutral to alkaline, ammonia contented decreased in the substrates. These changes were mostly recorded in the single poultry substrate, single pig substrate, mixture of poultry and sheep substrates, mixture of poultry and cow substrates, mixture of pig and sheep substrates where the highest fly larvae mass production were recorded. Fly larvae activity induced the conditions of aerobic microorganisms 'growth. The final product has granular structure and was suitable to be used as organic manure. For soil fertility replenishment the study recommends the mixture of pig and sheep substrates, mixture of poultry and sheep substrates, single pig substrate and mixture of poultry and cow substrates decomposed by Musca and Calliphora larvae during the three stages.

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