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Microbiological quality of poultry manure used in market gardening in South Benin and impacts on the hygiene of *Lactuca sativa* leaves

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Abstract

The composting of animal waste, particularly poultry manure, contributes to their hygienization and makes it possible to obtain fertilizers of satisfactory microbiological quality for agriculture. There are two types of composting, namely aerobic composting and anaerobic composting. The main objective of this research is to study the evolution of the pathogenic microbial load of poultry manure respectively after aerobic composting and anaerobic digestion. To do this, poultry manure collected on a farm in South was treated by the two composting processes. Then, the two types of humus obtained was used in parallel with the raw manure for the culture of *Lactuca sativa* (lettuces).To measure the effectiveness of both processes, samples of the raw manure and the humus obtained after composting were analyzed in the laboratory for the detection of mesophilic aerobic germs (GAMs), *Escherichia coli,* coagulase-positive *Staphylococcus, Clostridium perfringens* and *Salmonella* spp. Samples of lettuces were also analyzed. *Escherichia coli* loading decreased by more than 90% at the end of both processes, aerobic composting was particularly more effective than anaerobic digestion. At the opposite, the loads of coagulase-positive *Staphylococcus* and *Clostridium perfringens* increased significantly at the end of the two processes. The results showed that the composting of the manure before its use as a fertilizer for the cultivation of *Lactuca sativa* helped to obtain lettuces of better microbiological quality.

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Introduction

The biodegradable organic waste (vegetable materials, animal dejections, food-processing industry's products, muds of water-treatment plants, household waste, etc.) can be by biotechnological processes recycled and transformed, which represent a solution of choice to remedy environmental pollution. These processes contribute to the hygienization of waste and allow obtaining excellent grounds fertilizers for the farming. The waste recycling in agricultural purposes passes by the composting which is a process of decomposition of the organic matter by microorganisms in defined well conditions (Misra *et al.*, 2005). There are two types of composting: the aerobic composting and the anaerobic composting.

The aerobic composting takes place in the presence of a big quantity of oxygen and unfold in two phases: the active phase and the phase of maturation. During the process, aerobic microorganisms decompose organic matter and produce carbon dioxide (CO_2), ammonia, water and heat (Misra *et al.*, 2005). Achieving good quality mature compost depends on such factors as aeration of the pile, humidity and temperature (Misra *et al.*, 2005).

Anaerobic composting, also known as biomethanisation, is the degradation of the organic matter by microbial fermentation in a fuel gas (biogas) and a solid residue (digestate) more or less impoverished in organic matters (Saidi and Abada, 2007). Anaerobic microorganisms are at the heart of this process, which takes place in absence of oxygen in four phases: hydrolysis, acidogenesis, acetogenesis and methanogenesis (Monzambe, 2002; Saidi and Abada, 2007). The produced biogas is a mixture of methane (CH_4), carbon dioxide (CO_2) and other accumulated compounds (Misra *et al.*, 2005).

The biomethanisation of animal waste allows obtaining very high potential fertilizer for the amendment of the cultures but also some energy at a lower cost for domestic applications (cooking, lighting, heating etc.).

Thus, biomethane production is a better solution to alleviate the difficulty of supplying fossil energy (coal, oil, natural gas, etc.) in rural and landlocked areas (Tou *et al.*, 2001).

In Benin, poultry manure is widely used as fertilizer in market gardening. However, studies have shown that the use of raw poultry manure in market gardening has an impact on the microbiological and chemical quality of the vegetables produced (Dougnon, 2014; Kouakou, 2005). Thus, the Committee on the Development of Microbiological Criteria in Foods (CECMA) in Quebec mentioned: "fresh vegetables and fruits can be vectors of pathogenic microorganisms coming from organic fertilizers, contaminated irrigation water"(CQIASA, 2009). From then on, there isa public health problem bound to the hygienic quality of vegetables produced with poultry manure. So, in order to obtain healthy vegetables, the hygienization of organic waste, used as fertilizers in market gardening, is imperative (Derel and Aubert, 2008).

The objective of this research was to study the evolution of the pathogenic microbial load of poultry manure respectively after aerobic composting and anaerobic digestion.

Materials and methods

Sampling and analysis of poultry manure collected on a farm in South Benin

Further to a preliminary survey of poultry farming practices in the South Benin which took place from December 15th, 2014 till February 09th, 2015, a farm which met specific criteria for good husbandry practices, was selected to Pahou (municipality of Ouidah). After collecting manure on this farm, five (5) samples of about 500 g each were collected, stored in an insulating box at 4 ° C and transported to the laboratory for the search for GAMs(ISO 4833-1 : 2013), *E. coli* (ISO 16649-2 : 2001), *coagulase-positive Staphylococcus* (ISO 6888-2 : 2003), *C. perfringens* (NF V 08-056 : 1994) and *Salmonella* spp (ISO 6579 : 2002). The analytical methods used are accredited ISO/IEC 17025 by The Belgian Accreditation Body (BELAC).

Five additional samples of 500 g each were taken and analyzed for the measurement of total nitrogen (Kjeldahl), total phosphorus and bioavailable phosphorus (AOAC, 1997) in order to measure the fertilizer content of the manure.

Realization of aerobic composting and anaerobic digestion of the poultry manure

The manure thus collected was divided into two batches of approximately identical weight. Both manure lots were treated by aerobic composting and anaerobic digestion respectively. The work was carried out at the Center of Valorization of Waste into Renewable Energies and Agriculture (VWREA)of the University of Abomey-Calavi.

Aerobic composting

The manure was spread on a large tarpaulin placed on the ground. A swath of 3 meters in length, 2 meters in width and 20 centimeters in height was made by making two layers of manure. The two layers were watered one after the other and pressed down to facilitate the infiltration of the water to the depth of the pile and to stimulate the metabolic activity of the microorganisms.

The pile thus made was covered with a second tarp, then the turning and the watering were carried out every two weeks. The turning sallowed oxygenating the heap as completely as possible. The process lasted three months. The humus obtained was spread on a new tarpaulin and dehydrated in ambient air for two weeks.

Anaerobic digestion

The anaerobic composting of the manure was carried out with a mobile household metal digester with a capacity of about one (1) m^3composed of: a waste loading bar, a fermentation tank, a biogas outlet pipe, a flexible connection to the burner of a stove, an effluent discharge pipe, a pressure gauge, and discharge valves for the digestate and the biogas (Figure 1). The system is designed for continuous operation, ie after the initial period, the digester can be fed daily with animal, plants or domestic waste with water so that effluent and biogas are obtained daily.

The manure was introduced gradually into the loading boat and then homogenized with about one hundred and twenty-five (125) liters of water to make it completely pasty. The mixture was introduced into the fermentation tank by opening the substrate introduction valve. The fermentation lasted eleven (11) weeks. The digestate obtained was recovered by opening the outlet valve located at the base of the digester. The digestate was spread on a large tarpaulin and dehydrated in ambient air for two weeks. The methanogenic process is carried out in four steps by microbial populations with different metabolic functions (Table 1).

Sampling and analysis of humus from aerobic composting and anaerobic digestion

After dehydration in ambient air, respectively 5 samples of 500 g each, compost and digestate were taken in sterile bags placed in an insulating box at 4°C and taken to the laboratory for the search for GAMs, *Salmonella spp., E. coli,* coagulase-positive *Staphylococcus* and *C. perfringens*.

The content in fertilizing elements of the humus was also measured. So, 5 additional samples of 500 g each of compost and digestate were taken and transported to the laboratory for the determination of total nitrogen, total phosphorus and bioavailable phosphorus.

Culture of Lactuca sativa with the compost and the digestate

Sampling and microbiological analysis of the cultivation soil

The culture was made at the VWREA Center. Using a sterilized spoon, 5 samples of soil about 500 g each were taken sterile bags in a random way in the area reserved for the culture. They were placed in an insulating box at 4°C and transported to the laboratory for the detection of GAMs, thermotolerant coliforms, coagulase-positive *Staphylococcus*, *C. perfringens* and *Salmonella* spp.

Culture and microbiological analysis of lettuces

The lettuce nursery was planted on three growing blocks; each block was composed of three boards of

3.6 m², so there were a total of nine boards of lettuce. By the block randomization method, the boards were fertilized at each block with compost, digestate and raw manure from the initial farm located in Pahou. The boards were watered with drilling water available on the site of the VWREA center.

At maturation, three samples of about 500 g of fresh lettuces each were taken in sterile bags, respectively, on planks amended with compost, digestate and raw manure. For each sample, the leaves were taken randomly, at different locations on all the three boards with the same fertilizer.

The leaves were cut about 5 centimeter from the root, using a pair of sterilized scissors. The three samples were immediately stored in an insulating box at 4°C and then transported to the laboratory for the search for GAMs, *E. coli*, coagulase-positive *Staphylococcus*, *C. perfringens* and *Salmonella* spp.

Results

The results of all the microbiological analyzes carried out in this research were calculated according to the recommendations of the standard NF EN ISO 7218 of October 2007.

Table 1. Synthesis of the methanogenesis process (Mozambe, 2002).

Phases	Type de transformation	Some microorganisms involved
Hydrolysis	Depolymerization to monomers : - Polysaccharides → oses (glucose, fructose…) - protein→amino acids - lipids→fattyacids + glycerol.	Hydrolyticbacteria : *Salmonella typhi* *Salmonella gallinarum* *Escherichia coli* *Acetobacter xylinum* *Klebsiella pneumoniae*
Acidogenesis	Fermentation of monomersin: - Volatile fatty acids (acetic acid, propionic acid ...) - Alcohols (ethanol, etc.) - CO_2, H_2O	Acidogenicbacteria : *Lactobacillus brevis* *Lactobacillus fermenti* *Leuconostocmesenteroides Leuconostocpentosaceus* *Clostridium tetani* *Clostridium butyricum Thermobacteriumyoghurti*
Acetogenesis	Fementation acidogenesis products (other than acetate) in acetate, $H_2$and CO_2	Acetogenic bacteria : *Acetobacter xylinum* *Salmonella typhosa* *Salmonella pullorum* *Mycodermaaceti*
Methanogenesis	Formation of methane (CH_4) from reduction of $CO_2$by H_2offormateand methanol	Methanogenic bacteria: *Methanobacteriumformicum* *M. propionicum* *Methanococcusmazei* *M. venielli*

Microbiological quality and contents in fertilizing elements of the manure collected on farm

The raw manure contained very high bacterial loads. There were more than 3.10⁷ CFU (Forming Colony Units)/g of GAMs, more than 1.5.10⁵ CFU/g of *E. coli* and 3.8.10³ CFU/g of *C. perfringens* (Table 2). Coagulase-positive *Staphylococcus* loading was lower than 10 CFU/g. *Salmonella* was absent.

The contents of the manure in total nitrogen, total phosphorus and bioavailable phosphorus were respectively: 27880.2 ± 971.6; 15404.7 ± 72.6 and 1168.2 ± 121.8 mg/kg (Figure 2).

Microbiological quality and contents in fertilizing elementsof the compost and the digestate

The number of *E. coli* decreased considerably both after aerobic composting and after anaerobic digestion. It rose from more than 1.5.10⁵ CFU/g in the raw manure to 10² CFU/g in the compost and 1.1.10⁴ CFU/g in the digestate (Table 2).

Table 2. Evolution of the bacterial loads (CFU/g) in manure after the aerobic composting and the anaerobic digestion and the microbiological quality of the cultivation soil (GAMs: mesophilic aerobic germs; Coag+ Staph: coagulase-positive *Staphylococcus*).

	Manure	Compost	Digestate	Cultivation soil
GAMs	> 3.10^7	> 3.10^7	> 3.10^7	> 3.10^7
E. coli	> $1,5.10^5$	10^2	$1,1.10^4$	-
Coag+Staph.	< 10	>$1,5.10^5$	>$1,5.10^5$	< 10
C. perfringens	$3,8.10^3$	> $1,5.10^5$	> $1,5.10^5$	$4,7.10^3$
Thermotolerant coliforms	-	-	-	$3,9.10^4$
Salmonella spp.	Absence/25g	Absence/25g	Absence/25g	Absence/25g

So, there was a reduction of *E. coli* loading of at least 99.9% in the compost and at least 92.3% in the digestate. In order to better estimate the effectiveness of both composting processes, the decimal logarithms of the bacterial loads of the manure before and after composting were calculated (Table 3).

Table 3. Decimal logarithms of bacterial loads (log_{10}CFU/g) in manure after aerobic composting and anaerobic digestion.

	Manure	Compost	Digestate
GAMs	>7.5	>7.5	>7.5
E. coli	>5.2	2.0	4.0
Coag.+Staph.	< 1.0	>5.2	>5.2
C. perfringens	3.6	> 5.2	> 5.2

Unlike *E. coli*, manure loadings of coagulase-positive *Staphylococcus* and *C. perfringens* increased significantly at the end of both processes (Table 2). The number of coagulase-positive *Staphylococcus* increased from less than 10 CFU/g in the raw manure to more than $1.5.10^5$ CFU/g in the compost as well as in the digestate. The number of *C. perfringens* increased also by more than $1.5.10^5$CFU/g in both compost and digestate.

Table 4. Bacterial load (CFU/g) on lettuces produced on the soil of VWREA Center according to the fertilizers (GAMs: mesophilic aerobic germs; Coag+ Staph: coagulase-positive *Staphylococcus*).

	Lettuces		
	Manure	Compost	Digestate
GAMs	$1,8.10^7$	$1,1.10^7$	$6,9.10^6$
E. coli	$6,7.10^2$	80	$6,6.10^2$
Coag+Staph.	< 10	< 10	< 10
C. perfringens	$1,8.10^2$	$1,5.10^2$	50
Salmonella spp.	Absence/25g	Absence/25g	Absence/25g

The content of the manure in bioavailable phosphorus increased by 2012.6 mg/kg after the aerobic composting and by 1716.8 mg/kg after the anaerobic digestion.

On the other hand, the total nitrogen content decreased at the end of both processes,the decrease was greater in the compost, the decrease was more important in the compost (Figure 2).

Microbiological quality of the lettuces

The amount of GAMs on the lettuces cultivated with the compost and the digestate was lower than the amount of these germs on the lettuces cultivated with the raw manure (Table 4). There was a slight increase in *E. coli* loading on lettuces grown with digestate compared with to the load on lettuces grown with the raw manure. As for the quantity of *C. perfringens*, it increased slightly on lettuces obtained with the compost compared with its quantity on lettuces obtained with raw manure, while it decreased significantly on lettuces obtained with digestate. As regards to the number of coagulase-positive *Staphylococcus*, it was very low (<10 CFU/g) and did not vary as much on the lettuces obtained with the compost as on those obtained with the digestate.

Table 5. Comparison of the microbiological quality of lettuces obtained with compost with some microbiological criteria applicable to raw vegetable products ready for consumption.

	CFU/g				
	GAMs	*Coag+Staph.*	*C. perfringens*	*E. coli*	*Salmonella* spp.
Lettuces produced with compost (n = 1)	$1,1.10^7$	< 10	$1,5.10^2$	80	Absence/25g
Microbiological criteria (Guiraud, 2003 ; Grand-Duchéde Luxembourg, 2015 ; CQIASA, 2009)					
Fresh fruits salads					
Order of 22 March 1993[a] (n = 5)	-	< 10	< 10	< 10	Absence/25g
Standard of desserts[b] (n = 5)	-	10^2	-	-	
Products of IV[th] range*					
CNERNA[c] (n = 5)	$< 5.10^6$	-	-	$< 10^2$	Absence/25g
CECMA[d] (n = 5)	10^7	10^2		10	Absence/25g

[a]Order of 22 March 1993 on the hygiene rules applicable to plants and preparations of plants ready for human consumption (France).

[b] Internal standard related to desserts (Grand-Duché de Luxembourg)

[c] National Center for Studies and Recommendations on Nutrition and Food(France).

[d] Committee on the Development of Microbiological Criteria in Foods (Quebec).

*Products packaged, raw, fresh, ready for consumption and having been peeled or cut.

Discussion

Microbiological quality of the compost and the digestate and effectiveness of both composting processes

The results of the analyzes showed that both composting processes were effective because the amount of *E. coli* in the manure decreased by more than 90% both after aerobic composting and after anaerobic digestion. Aerobic composting was even more effective than anaerobic digestion because the decrease of *E. coli* loading was more significant in the compost than in the digestate (Table 2). This result confirms Misra *et al.* (2005) who consider that aerobic composting is more efficient and useful than anaerobic composting for agricultural production. The commensal germs, namely *E. coli* and enterococci, are acceptable indicators of the effect of composting and are used to assess the effectiveness of composting hygienisation (Moletta and Guillou, 2013). Exceeding their limit value does not lead to non-compliance. In the context of our study, only *E. coli* was searched and counted.

At the opposite, the quantities of coagulase-positive *Staphylococcus* and *C. perfringens* in both compost and digestate were significantly higher than the initial quantities contained in the raw manure.

On the one hand, at the end of the aerobic composting, mesophilic germs recolonize the pile but the microbial activity is totally reduced in the mature compost; on the other hand, pathogens are not affected during the anaerobic composting because this process is carried out at low temperatures (Misra *et al.*, 2005). This confirms the increase of the amounts of pathogenic mesophilic germs (coagulase-positive *Staphylococcus* and *C. perfringens*) in both compost and digestate.

Moisture may also justify high amounts of coagulase-positive *Staphylococcus* and *C. perfringens* in the compost. Indeed, the process of aerobic composting was disrupted by the great rainy season of May and June. During this period, the pile had to absorb much water by infiltration. However, compost should have a water content of 40 – 65% (Misra *et al.*, 2005). Too high humidity results in a drop in temperature within the pile, which prevents the destruction of pathogens. It should also be noted that the experiments were carried out with non-sterile equipment in a microbiologically uncontrolled working environment, thus considering these working conditions as a potential source of contamination of the final products.

Fig. 1. Family metal digester.

Contents in fertilizing elements of the compost and the digestate

Unlike nitrogen, phosphorus levels increased in compost and digestate because there is no gas production during its biogeochemical cyclization. This result confirms those of Dougnon *et al.* (2014) for their research on the anaerobic digestion of poultry manure Note that bioavailable phosphorus is the most useful form of phosphorus for plant survival because it is the part of the total phosphorus, directly assailable by plants and necessary for their nutrition (Beaudin *et al.*, 2008).

Microbiological quality of lettuces cultivated on the soil of VWREA Center

The lettuces produced with compost and digestate were less contaminated in GAMs than those that were produced with raw manure, which shows that the composting of poultry manure before their use in market gardening is useful for obtaining vegetables of better hygienic quality. The increase of the level of contamination observed for *E. coli* and *C. perfringens* on the lettuces produced with either compost or digestate can be explained by a potential contamination by irrigation water or the soil of cultivation.

At present, there are a few data on microbiological criteria for raw plant products (Guiraud, 2003), in particular for vegetables that are eaten raw such as *Lactuca sativa*. According to CECMA in Quebec, "untransformed raw vegetables and fruits are not likely to allow the growth of pathogenic microorganisms when they keep on their integrity" (Centre québécoisd' inspection des aliments et de santé animale, 2009).

The available data mainly concern transformed raw fruits and vegetables (products of 4th range) and fresh fruits salads (Guiraud, 2003). By assimilating *Lactuca sativa* leaves to this category of products, it is possible to interpret the results of the microbiological analysis of lettuces obtained in the context of our study on the basis of some microbiological criteria of various legislations.

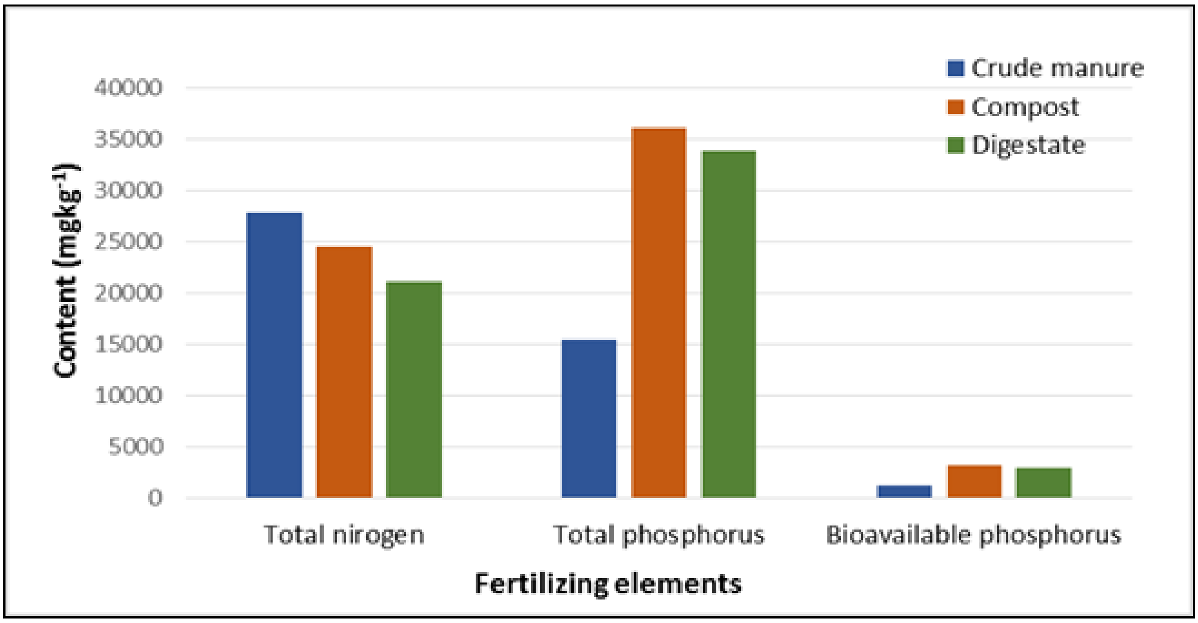

Fig. 2. Content of the manure in fertilizing elements (mg/kg) before and after aerobic composting and anaerobic digestion.

Example of interpretation of analysis results of lettuces produced with compost (Table 5)

Considering the number of GAMs, the quality of these lettuces is very unsatisfactory (much higher value) with regard to that recommended by the National Center for Studies and Recommendations on Nutrition and Food (CNERNA) in France and the CECMA (Quebec) for the products of 4th range. Therewith, the number of *C. perfringens* on these lettuces is unsatisfactory in view of the Order of March 22nd, 1993 for fresh fruits salads.

As regards the quantity of *E. coli*, the quality of the lettuces is unsatisfactory with regard to the Order of March 22nd, 1993 and the recommendation of the CECMA but it is in conformity with what is proposed by CNERNA.

The number of coagulase-positive *Staphylococcus* on the lettuces complies with both the recommendations of the Order of March 22nd, 1993 and the CECMA.

Conclusion

Both composting techniques were effective due to the significant decrease in the number of *E. coli*. However, aerobic composting was more effective than anaerobic digestion.

The hygienisation of the manure before its use as a fertilizer for the cultivation of *Lactuca sativa* has made it possible to obtain, globally, lettuces of better microbiological quality. However, the increase in the level of contamination observed for certain germs on lettuces grown with compost or digestate suggests a potential contamination by the water used for irrigation or the soil.

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