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Hygienization assessment during heap co-composting of Turkey manure and olive mill pomace

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Abstract

This study aimed to investigate the co-composting time effectiveness as well as the effect of the initial Carbon/Nitrogen ratio (C/N)i variation on the hygienization of olive pomace and turkey manure. Six different heaps, at 3 levels of (C/N)i ratios: 20, 22 and 28, were installed and monitored during 6 months and assessed at three steps: At the beginning, the end of thermophilic-phase and the end of curing-phase. The microbial monitoring concerned 5 microbial pathogens contents, used as hygiene microbial indicators, namely: Sulphite-Reducing Anaerobes (SRA), Escherichia Coli (E. Coli), Total Aerobic Mesophilic Flora (TAMF), Staphylococci, and *Salmonella* spp. Initially, the mixtures showed high TAMF and *Staphylococci* loads. Meanwhile, SRA and E. coli populations were relatively low and *Salmonella* spp. was not detected. The microbial assessment showed a significant effect of composting time on the reduction of pathogens load, except for SRA where its population has increased significantly, while the $(C/N)_i$ had a non-significant effect on pathogen content of the end-product. The final values expressed as colony-forming unit per gram (CFU g^{-1}), were as follow: Sulfite-reducing Anaerobes (≤3.1 x 10^3 CFU g^{-1}), E. Coli germ used as an indicator of faecal contamination (<4 x 10^1 CFU g^{-1}), Total aerobic mesophilic flora (≤1.4 x 10^6 CFU g^{-1}), *Staphylococci* (<10 CFU g^{-1}) and non-detection of *Salmonella* spp. Finally, the seed germination tests were carried out on three different seeds: lentils (Lens culinaris), barley (Hordeum vulgare) and durum wheat (Triticum turgidum) showed that the use of the compost extract is favourable for seed germination with germination index (GI%) values exceeding 85%. These results confirm the non-phytotoxicity and maturity of the composts.

Keywords: Sanitation, pathogens, poultry manure, microbial hazard, germination index, olive by-products.

Introduction

Agro-food industries generate a considerable amount of organic wastes. They can be recycled as organic amendments after biological treatments (Tortosa et al., 2019). Nowadays, the rapid increase of biowaste production has become one of the most crucial issues in most countries around the world (Azim et al., 2017). Besides, intensive agricultural activities lead to soil fertility depletion, worsens soil erosion, and causes organic matter content reduction (Ramli et al., 2020). The olive crop is one of the main cultivation in Morocco and also in several Mediterranean countries and its socio-economic role is well known. Thus, olive production causes a serious environmental issue through the by-products generated from olive factories (Toledo et al., 2020). The three-phase system used for olive oil production generates huge amounts of by-

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products, namely olive mill wastewater (OMW) and olive mill pomace (OMP) within few months in a year according to Roig et al. (2006).

On the other hand, throughout the last decades, the poultry livestock has increased significantly in most countries, which has led to an increase in poultry manure production and has increased considerably the amount of all organic solid by-products and wastewaters (Assess et al., 2019; Toledo et al., 2020). According to Aboutayeb (2015), in the Chaouia-Ouardigha region in Morocco, quite 300,000 tons of turkey manure (TM) are produced yearly. The number of turkey livestock farms is up to 220 production units and the production capacity exceeds 5 million turkeys per breeding cycle (Aboutayeb, 2015). These intensive livestock systems cause ecological problems due to the production of huge amounts of manure, the spread of microbial pathogens and the release of putrid odours (Heinonen-Tanski et al., 2006).

While olive pomace is not usually associated with the risk of microbial pathogens, poultry manure could be a source of microbial hazard when it is spread as an organic amendment without former treatment. It contains several human pathogens species like *Escherichia Coli*, *Clostridium, Listeria and Salmonella* (Chen et al., 2014). Moreover, it provides favorable conditions for the proliferation of microbial pathogens, which worsens environmental pollution (Li et al., 2016).

In this regard, according to Bustamante et al. (2008), composting is defined as a biological process occurring in an aerobic environment leading to organic materials stabilisation and heat production. Composting has been presented as a promising technology and environmentally friendly technique to manage and recycle these biowastes, to obtain a quality compost used as an organic amendment soil fertility improvement (Azim et al., 2018; Assess et al., 2019). Among the various biological treatments, composting is perceived to be among the most promising practices due to its low cost and effectiveness to produce stable organic amendments according to de Mendonça Costa et al. (2016) and Soobhany et al. (2017). These end-products, called composts, could be used as organic amendments able to enhance soils physicochemical and biological properties (Chowdhury et al., 2013; Tortosa et al., 2019). Composting process, as biological treatment, could be used for biowastes processing to reduce its pathogenic potential (El Fels et al., 2014; Assess et al., 2019). Actually, it is a widely accepted technique for organic waste recycling into a stable organic material, with low pathogens loads and phytotoxicity. Compost is applied as an organic fertilizer and soil amendments to enhance soil fertility parameters (Huang et al., 2006; Toledo et al., 2020) and increase plant growth and yield production (Bustamante et al., 2008).

Hence, the biowaste reuse as compost on agricultural soils can play a vital role in increasing the sustainability of agricultural practices, especially in Mediterranean countries where soil organic matter (SOM) is generally low as mentioned by Assess et al (2019). Indeed, soils of semi-arid rainfed areas, such as those of the West-Asia and North-Africa regions have less than 1.5% SOM content (Azim et al., 2017; Aboutayeb et al., 2020).

Depending on the composting process monitoring and the raw material origin, composts may also contain pathogenic microorganisms (Bustamante et al, 2008). Instead, the raw manure or immature compost spreading can induce pathogenic microorganism dissemination according to Millner et al. (1994) and Beffa et al. (1996) such as *Salmonella* spp., *Listeria monocytogenes* and *E. coli*, (Zhao et al., 1995; Islam et al., 2005; Soobhany et al., 2017). Moreover, the composting process improperly monitored can be a vector of several pathogens species initially present in raw organic waste or those resulting from the risk of re-proliferation during composting (Chen et al., 2014). Hassen et al. (2001) and Bustamante et al. (2008) have registered the proliferation of Shigella, Enterobacter, Yersinia and Streptococci which could cause infections diseases for farmers and compost handlers. . Actually, growing demand for sanitized compost is observed which reflects the increased interest in the food safety and environmental issues (Pandey et al., 2016; Soobhany et al., 2017). In certain cases, the remaining pathogenic organisms in a compost pile has been attributed to 3 main factors: inequal heating temperatures among different parts of the compost heap: The surface, the middle and the bottom of the piles (Aboutayeb, 2015); Mixtures inadequately homogenized as mentioned by Elving et al. (2010), and cross-contamination due to infected working tools (Soobhany et al., 2017). Hygiene microbial indicators, such as faecal coliforms, *E. coli* and streptococci are generally monitored during the composting process to ensure compost quality production (Bustamante et al., 2008; Aboutayeb, 2015). The subsistence of pathogenic populations in compost piles remains less explored (Soobhany et al., 2017). Faecal coliforms are generally associated with the animal faeces such as poultry manure (Aboutayeb et al., 2013); this is why regulations have adopted fecal coliforms, especially in *E. coli*, as an indicator of potential fecal contamination to assess the hygienic quality of the final compost (Soobhany et al., 2017). Although the composting process is potentially effective to reduce pathogens loads and thus producing a sanitized

composts (Soobhany et al., 2017), data on the relationship between pathogen reduction and composting duration remains unsettled and needs more attention. This study aims to assess the effect of the initial C/N ratio and Treatment-time of co-composting on the microbial characteristics of the final compost concerning different human pathogens and microbial groups used as hygienic microbial indicators.

Material and Methods

Raw materials

The turkey manure used to carry out this study was collected and transported to the composting experiment site from three Turkey farms located in the immediate vicinity of Settat province in North-West of Morocco. The composting site is located in the experimental station of Sidi Elaidi (altitude 230 m, 33.17° N, 7.40° W) belonging to the National Institute of Agricultural Research (INRA-Morocco). We have taken six composite samples for microbial analysis. The Olive mill pomace (OMP) was collected from a three-phase artisanal crushing unit (Maasras) in the Settat region. The durum wheat straw was used as a bulking agent for the composting process. Six heaps were prepared to obtain the initial C/N of 20, 22 and 28 in duplicate (2 heaps for each ratio) (Table 1). C/N was calculated using the formula OC(%)/TN(%) where OC(%) = OM(%)/1.73 (Table2).

Table 1. Composition (weight/weight) and (C/N)i of different mixtures

Heaps	Durum wheat straw	Turkey manure	Olive mill pomace	C/N	Height (m)	Width (m)	Length (m)
H1	10.0%	26.4%	63.6%	20	1.2	1.3	1.5
H2	10.0%	26.4%	63.6%	20	1.2	1.3	1.5
H3	60.0%	10.0%	30.0%	28	1.5	1.4	1.8
H4	60.0%	10.0%	30.0%	28	1.5	1.4	1.8
H5	20.0%	20.0%	60.0%	22	1.6	1.4	1.8
H6	20.0%	20.0%	60.0%	22	1.6	1.4	1.8

Composting process

Olive mill pomace and turkey manure were added to the durum wheat straw and the heaps were moistened if necessary and composted in aerobic conditions. The experiment of composting is carried out for six months. During the composting process, the heaps have been manually turned. Heap temperatures monitoring was carried out using a compost thermometer. A sample from each heap was collected in sterile plastic bags to serve for physicochemical and microbial analysis. The Organic carbon, organic matter and nitrogen contents properties of composts, at the initial and final time, are shown in Table 2.

Table 2. Physicochemical properties of composts at the initial and final time (TNK: total nitrogen, OM: organic matter, C/N: Carbon to nitrogen ratio).

	H1		H2		H3		H4		H5		H6	
	Initial	Final	Initial	Final	Initial	Final	Initial	Final	Initial	Final	Initial	Final
TNK (%)	1.82 ± 0.15	2.26 ± 0.03	1.84 ± 0.08	2.77 ± 0.06	1.36 ± 0.06	2.58 ± 0.03	1.21 ± 0.02	2.81 ± 0.01	1.65 ± 0.12	2.82 ± 0.05	1.63 ± 0.07	2.66 ± 0.00
OM (%)	61.72 ± 0.90	43.66 ± 0.98	58.96 ± 0.70	58.58 ± 1.31	66.37 ± 0.13	54.1 ± 1.31	58.96 ± 0.31	64.76 ± 1.75	62.65 ± 0.58	54.95 ± 0.11	59.04 ± 0.39	50.31 ± 2.30
C/N	19.73 ± 1.88	11.26 ± 0.4	18.65 ± 0.59	12.26 ± 0.54	28.34 ± 1.36	12.15 ± 0.43	28.17 ± 0.22	13.38 ± 0.31	22.10 ± 1.76	11.30 ± 0.22	21.02 ± 0.77	10.97 ± 0.50

Microbial Analysis

Microbial analyses were performed on the enumeration of 5 microorganisms considered as hygienic microbial indicators. The assessment of microorganism loads was expressed in colony-forming units per gram (CFU g^{-1}). The samples were analyzed in three stages: S1 (at the beginning of composting), S2 (after 3 and 6 weeks of composting for heaps 5,6 and heaps 1,2,3,4 respectively) and S3 (at the end of the composting experiment). The Total Aerobic Mesophilic Flora (TAMF) density was determined using Plate Count Agar (PCA) medium and incubation at 30° C for 72h (ISO 4833-1:2013). *E. coli* was assessed using the tryptone-bile-glucuronide medium, incubation at 44±1°C for 18 h to 24 h. (ISO 16649-2:2001). *Staphylococcus aureus* load was determined using rabbit plasma fibrinogen agar medium, then incubation at 37° C for 24h (ISO 6888-2:1999). Sulfite-reducing bacteria (characterized by typical black coloured colonies) were incubated under anaerobic conditions on agar plates using an iron-sulfite medium, then incubated at 37◦C ± 1°C for 24h to 48h (ISO 15213:2003). Finally, the determination of *Salmonella* presence was carried out, in 25g sample inoculated to buffered peptone water and incubated at 37 °C ± 1°C for 18 h ± 2 h, isolated then identified and confirmed following the protocol of ISO 6579-1:2017/AMD 1:2020.

Phytotoxicity test and germination index (GI)

The phytotoxicity test is based on the principle of the compost aqueous extract phytotoxicity towards the tested seeds. It involves placing the seeds of three different species (Durum wheat (*Triticum turgidum*), Barley (*Hordeum vulgare*) and Lentil (*Lens culinaris*), in a series of Petri dishes with filter paper impregnated with increasing doses of the compost extract: 25%, 50%, 75% and 100% (v/v) (three repetitions for each treatment). Another set of control (without compost aqueous extract) is prepared with distilled water and its germination index is considered as 100% for relative comparison with the treatments. The Petri dishes are placed in the germination chamber for 5 days at a temperature of 25°C and relative humidity of 85 to 90%. After 3 days the number of seeds germinated per petri dish was determined. The phytotoxicity levels of the compost extracts were determined according to a standard method (Zucconi et al., 1981). Index Germination was calculated using the following formula:

$$IG = \frac{(\text{Nmgg} * \text{Lr})}{\text{Nmggt} * \text{Lrt}}$$

Where
Nmgg : Number of germinated seeds;
Lr : Average length of the root;
Nmggt : Number of germinated seeds of the control;
Lrt : Average length of the witness root.

Statistical Analysis

The effect of the factors studied (C/N ratio and Time) on the evolution of microbial populations was evaluated by ANOVA 2 and Tukey's test. The results were carried out using SPSS software, Version 20.

Results and Discussion

Temperature monitoring

Temperature is one of the major parameters to assess the progress of the composting process as it indicates the rate of microbial activity (Manu et al., 2019). Many authors consider 45° C as the temperature limit between the mesophilic and thermophilic phase during composting (Albrecht, 2007; Pujol, 2012). A temperature above 45° C can hygienize the heaps by reducing pathogens loads (Aboutayeb et al., 2013). All the heaps have recorded (Figure 1) thermophilic phases with maximum temperatures of 62, 57.3, 60.1, 59.6, 56.6 and 61.3° C for heaps 1 to 6 respectively, indicating organic matter biodegradation. The short periods of the thermophilic phase characterized the heaps with a lower proportion of straw and low initial C/N (H1 and H2) who reached the thermophilic phase faster due to their high manure content, and therefore high initial microbial load. After thermophilic phase, the six heaps have entered the maturation phase where the temperature tends to the ambient one indicating the end of the composting process. There is a similarity between the temperature curves of the heaps with the same composition (H1, H3 and H5 with H2, H4 and H6 respectively).

Evolution of the microbial parameters

At the start, the initial mixtures showed a high load of *Staphylococci* and TAMF populations, due probably to non-compliance with good hygiene practices in the *Maasras* (Rouas et al., 2015) and given that TM is considered as natural host for many microbes (Bustamante et al., 2008). However, all the mixtures recorded no presence of *Salmonella* even if the manure is a natural host, which proves good hygiene control in the livestock house (Aboutayeb, 2015).

Sulphite reducers Anaerobes (SRA)

Several studies have focused on the pathogenic density decay as one of the most important factors through composting process according to Gale (2004) or after compost spreading on agricultural soils, for healthy risks assessment (Soobhany et al., 2017). The presence of Clostridium bacteria, a pathogenic SRA germ, can be used as a suitable indicator for other faecal pathogens (Bustamante et al., 2008). Except for H2, all the heaps recorded an increase at S2 of composting time (Table 3). Then, H1, H3, H4 and H6 recorded a reduction at the end of composting while H2 and H5 recorded a slight increase in SRA by the end of the process. The statistical test shows that only the composting time factor has a significant effect on the increase in SRA load but only between the S1 and S2 composting stages. The final reduction was not significant which is unusual for SRA considered as a strict anaerobic germ. It could be explained by the presence of certain SRA species that tolerate oxygen presence and able to generate ATP in an aerobic environment, or capable to reduce nitrates (Loubinoux, 2001). The final reduction of SRA, even if it is not significant, can be explained by the scarcity of labile organic matter during the curing phase, the aerobic conditions and the presence of nitrates (Aboutayeb, 2015).

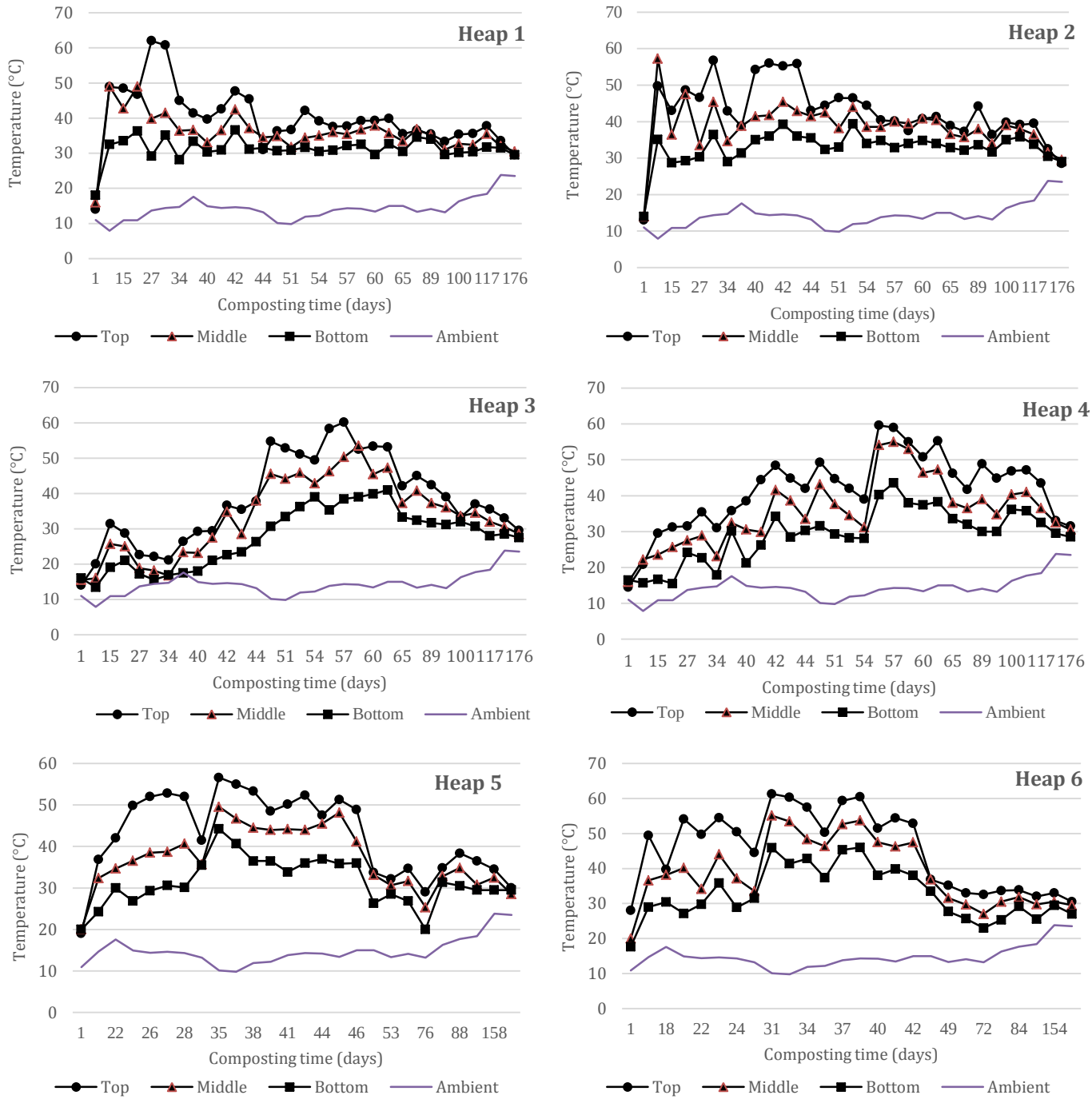

Figure 1. Temperature evolution during co-composting of olive pomace with turkey manure

The SRA loads in all heaps remain above 10^3 CFU g^{-1}. This result is consistent with those found by Bustamante et al. (2008) who mentioned that there is no enough hygienization in composting piles to ensure the total elimination of *Clostridium*. This result could be explained by the fact that *Clostridium* bacteria show resistance to several adverse conditions and are among the heat resistant bacteria as mentioned by Juneja and Marmer (1998) and Payment (1999). However, the distribution of SRA makes challenging the production of free-*Clostridium* composts even in aerobic conditions (Juneja et al., 2003; Bustamante et al., 2008). This resistance could be explained by spores ability to survive in harsh conditions and the remaining anaerobic spaces leading to SRA growth (Böhnel and Lube, 2000; Jones and Martin, 2003; Pourcher et al., 2005). These results are better than those found by Bustamante et al. (2008) who found higher levels of SRA in turned piles, in general exceeding 10^4 CFU g^{-1}. These findings may be due to higher temperature values, during the thermophilic phase, reached in turning heaps and thus a more effective elimination of SRA have been recorded.

Table 3. Monitoring of SRA and E.Coli content during the composting process. (S1 (at the beginning of composting), S2 (after 3 and 6 weeks of composting for heaps 5,6 and heaps 1,2,3,4 respectively) and S3 (at the end of the composting experiment)

Heaps	SRA			E.Coli		
	S1	S2	S3	S1	S2	S3
H1	2.4×10^2	2.10×10^4	2.00×10^3	2.0×10^2	1.90×10^4	<10
H2	2.4×10^2	1.0×10^2	2.40×10^3	2.0×10^2	3.30×10^5	<10
H3	9.0×10^2	4.80×10^4	1.70×10^3	1.0×10^2	1.30×10^4	<10
H4	9.0×10^2	1.60×10^4	2.90×10^3	1.0×10^2	7.8×10^2	<10
H5	2.0×10^2	1.60×10^3	2.40×10^3	1.3×10^2	1.10×10^4	<10
H6	2.0×10^2	1.10×10^5	3.10×10^3	1.3×10^2	5.70×10^5	<40

Escherichia coli

E. coli as an emerging pathogen is the main faecal coliform microorganism. It is usually considered as the most important pathogen to investigate in all processes that use or integrate faecal materials (Déportes et al., 1998; Hess et al., 2004; Bustamante et al., 2008). The presence of coliform bacteria reflects the hygienic quality level of soil and water in the environment. Its utilization as a hygienic indicator could bring several benefits due to the high detection frequency and easy revelation in faecal materials compared to other pathogens (Hassen et al., 2001).

Generally, the monitoring of the composting process showed the same evolution for both *E.coli* and fecal coliforms bacteria (Le Minor, 1984). The $(C/N)_i$ factor had a non-significant effect on *E.coli*; while the composting-time factor recorded a significant effect. *E.coli*, considered a thermotolerant germ, is not considered heat-resistant. Its growth limit temperature is 45.5°C (Vernozy-Rozand and Roze, 2003). At composting time S2 (Table 3), *E.coli* increased in all the heaps, given the favourable temperature because all the heaps were still in the mesophilic phase except H1 where the temperature exceeded 45° C but for just one day.

At the end of composting, *E.coli* was almost eliminated in all the heaps. *E.coli* was removed in H1 even though its temperature was < 36.7° C from S2 until composting end, which may be in agreement with the findings of Larney et al. (2003) who concluded that more than 99.9% of *E. coli* was removed, during the first 7 days of composting, at temperatures ranging from 33.5 to 41.5°C. The thermophilic temperature/time pairs of H1 to H6 were (43.5-45.8°C/4 days separately), (43.6-46.6°C/4 days), (43.7-47.1°C/14 days), (47-51.3°C/6 days), (42.7-50.1°C/11 days) and (44.7-54.1°C/14 days) respectively. These lethal time/temperature pairs are in agreement with other studies findings: Total destruction of *E.coli* in manure at 45°C during 72 hours of composting (Lung et al., 2001), and 7-14 days at 67°C as stated by Johannessen et al. (2005). E. Coli regrowth was observed during the first weeks (between S1 to S2 time of composting) which could be usually attributed to recontamination phenomena or explained by the insufficiency of compost self-heating and composting time (Soobhany et al., 2017).

The reduction of *Escherichia coli* loads used as a faecal-contamination indicator was significant in all final heaps. The *E. Coli* population is compliant with the recommended limit (10^3 CFU g^{-1}) indicating the heap co-composting efficiency (Ros et al., 2006; Aboutayeb, 2015). This significant decrease could be explained by both a high temperature occurring in the heaps and aerobic conditions (Semenov et al., 2011).

This decay was likely the result of the high temperatures (thermophilic phase between 43.5°C and 54.1°C in all the heaps). Similar conclusions have been recorded by Hess et al (2004), who mentioned a decline, then an increase of *E.coli* population at 50°C. Other studies highlighted that even with temperatures reaching up to 66°C, the elimination of *E.coli* is not complete. According to De Bertoldi et al (1983) and confirmed by Assess et al. (2019), during the thermophilic stage, the recommended temperature is ranging between 40 and 65°C and temperatures above 55°C are required to eliminate coliforms considered as thermotolerant microorganisms. An important decrease in the *E.coli* population was recorded after the thermophilic phase in all the heaps. This finding complies with several previous works that have shown the ability of the composting process to eliminate E. coli (Mainoo et al., 2009; Aira et al., 2011).

TAMF

The effectiveness of the composting-time factor was high on the reduction of TAMF, especially between S2 and the end of composting (Table 4). Initially, all the heaps showed a high density of TAMF. At S2 time of composting, the density of TAMF increased for all heaps, which is consistent with the work of El Fels (2015) who reported that mesophilic bacteria growth continues even at 50°C. Then, TAMF was significantly reduced in the mature composts. This decrease was mainly due to the temperature increase during the thermophilic

phase and the unfavourable conditions in the heap essentially due to the labile organic matter depletion'' (Kalamdhad and Kazmi, 2009; Aboutayeb et al., 2013). The final density of TAMF which still quite important could be explained by its re-proliferation during the maturation phase characterized by the favorable temperature conditions for mesophilic microflora growth (El Fels et al., 2015).

Strict monitoring, especially of the temperature profile, should be performed to reduce contamination risks due to pathogens high loads essentially when raw animal material, considered as a natural host, was used. The outcomes of other studies were consistent with these results by the fact that mesophilic bacteria were predominant at the beginning of the co-composting process and their population remain around 10^5 and 10^6 CFU g^{-1} during the cooling phase (Hachicha et al., 2009; Assess et al., 2019).

Table 4. Monitoring of TAMF, *Staphylococci* and *Salmonella* content during the composting process (ND: not detected. D: detected)

Heaps	TAMF			*Staphylococci*			*Salmonella*		
	S1	S2	S3	S1	S2	S3	S1	S2	S3
H1	3.54×10^6	2.50×10^7	7.60×10^5	4.49×10^6	9.80×10^6	<10	ND	ND	ND
H2	3.54×10^6	2.00×10^7	2.70×10^4	4.49×10^6	<10	<10	ND	ND	ND
H3	1.43×10^6	1.50×10^7	1.40×10^6	1.70×10^6	<10	<10	ND	ND	ND
H4	1.43×10^6	1.60×10^7	2.10×10^5	1.70×10^6	2.10×10^6	<10	ND	ND	ND
H5	2.86×10^6	3.20×10^6	1.50×10^5	3.40×10^6	<10	<10	ND	ND	ND
H6	2.86×10^6	2.00×10^7	2.00×10^5	3.40×10^6	<10	<10	ND	D	ND

Staphylococci

Staphylococci is considered an optional aero-anaerobic microorganism widely known as one of the main causes of food biological hazards such as toxic infections (Vernozy-Rozand et al., 2004; Bustamante et al., 2008). *Staphylococci* growth occur under a large pH interval (4-9.3), requires temperature ranging between 7°C and 46°C and water activity (Aw) above 0.84 in aerobic conditions (Lamprell, 2003). Table 4 shows that in all heaps, the C/N factor has a no-significant effect on *Staphylococci* content while composting time has reduced significantly its load. It was almost eliminated by the end of the composting period (beneath 10 CFU g^{-1} for all the heaps).

The number of *Staphylococci* increased during the bio-oxidative phase in H1 and H4 then decreased at the maturation phase in all the heaps (beneath 10 CFU g^{-1}). These results could be explained by the temperature evolution (heating and cooling) in the bio-oxidative phase and scarcity of labile organic matter in addition to the competition with other microorganisms essentially fungi (molds and yeasts) present in the heaps. These results are in agreement with those found by Bustamante et al. (2008) where levels of *Staphylococci* detected were less than 3 CFU g^{-1} of compost. Some authors report that pathogens species could be inhibited by several compounds contained in the heap such as organic acids, ammonia and flavonoid compounds even though temperature remains the most effective factor (Ait Baddi et al., 2004; Hachicha et al., 2009).

These results are better than those found by Aboutayeb (2015) who concluded that the population of *Staphylococci* was significantly reduced through composting time; however, the remaining density (slightly higher than 10^4 CFU g^{-1}) could be due to the ubiquitous character of *Staphylococci*. Despite successful composting, the sanitary hazard remains probable due to a potential staphylococcus regrowth, especially in the peripheral parts (Albrecht, 2007; Sidhu et al., 2001; Aboutayeb et al., 2013).

Salmonella

Salmonella is a pathogenic bacteria of high concern in farming (Jamieson et al., 2002). It is related directly to the compost hygienic quality according to Brinton Jr and Droffner (1994) and Yanko et al. (1995). The risk related to this pathogen is increased because of its fast growing and ubiquitous presence (Hassen et al., 2001).

Several studies have concluded the possibility of the presence of *Salmonella* during biowaste composting even if the required time-temperature pairs were reached (Pourcher et al., 2005; Millner et al., 2014). As mentioned by Bustamante et al. (2008), *Salmonella* was detected in all animal biowaste which explains its presence in the composting pile containing poultry manure as a raw material. Even though *Salmonell*a is not among thermotolerant microorganisms, it could persist at a high temperature exceeding 50° C (Droffner et al., 1995).

The present work showed that *Salmonella* was completely absent in all the heaps by the end of the co-composting process (Table 4). This result is in agreement with those found by Soobhany et al. (2017) who concluded that *Salmonella spp.* decreased below the detection limit (Less than 1 most probable number per sample of 4 g). Similarly, it is consistent with the result of Aboutayeb (2015) who concluded the non-

detection of *Salmonella* in all the heaps. In the sixth heap (H6), *Salmonella* was only detected at S2 of composting (Mesophilic phase). This finding is consistent with other studies which demonstrated that *Salmonella spp*. was able to regrow in compost, windrows as a post-contamination even if the composting process was well monitored (Erickson et al., 2010; Soobhany et al., 2017). The reason for the regrowth could be explained by the lack of homogeneous temperature profile in the heap or due to recontamination occurring through compost handling (PereiraNeto et al., 1986; Gerba et al., 1995). Other factors could also influence *Salmonella* growth particularly, nutrient availability and moisture content (Bustamante et al., 2008). As a consequence, the production of *Salmonella* free compost requires an effective and efficient composting process monitoring to avoid pathogens regrowth (Soobhany et al., 2017).

Germination Index (GI)

In order to assess the compost maturity, the phytotoxicity test is one of the most important criteria used for this purpose (Bargougui et al., 2019). It allows to state reliable conclusions about the potential importance of the end-product, compost, to be used as an alternative organic fertilizer on agricultural crops. The final composts were assessed to reveal their phytotoxic potential, through the germination indexes (GI%) determination. Seeds of 2 cereals and a legume were used, Durum wheat, Barley and Lentils respectively.

The compost extracts were prepared from the six mature compost samples obtained from the studied process once finished. The compost extracts recorded quite good seeds germination and the recorded results were as follow: 101,42% ± 20,32%, 112,22% ± 15,74% and 92,29% ± 18,43% for lentils, durum wheat and barley seeds respectively, thus indicating both the absence of any phytotoxic effect and so the maturity of the olive pomace and turkey manure final co-compost (Table 5).

Table 5. Germination index (GI) of lentils, durum wheat and barley at different doses of compost extract (v/v)

	Lentils	Wheat	Barley
GI 25%	99,70% ± 35,84%	124,37% ± 2,69%	95,90% ± 36,03%
GI 50%	116,94% ± 17,61%	109,11% ± 35,59%	102,60% ± 11,47%
GI 75%	84,27% ± 3,38%	103,09% ± 18,27%	93,43% ± 9,25%
GI 100%	104,76% ± 24,47%	112,31% ± 6,42%	77,25% ± 16 ,99%
GI average (%)	101,42% ± 20,32%	112,22% ± 15,74%	92,29% ± 18,43%

GI value exceeding 80% reflects the non-phytotoxicity effect of mature compost according to Hachicha et al. (2006) and Francou (2005). These high seeds germination indexes values could be due to the high quality of the obtained compost and seeds resistance to residual phytotoxic compounds indicating consistency with previous studies (Bargougui et al., 2019).

Conclusion

From a hygienic point of view, co-composting of TM and OMP could remarkably decrease, over 6 months follow-up, the pathogen loads in the end-product especially for TAMF, sulfite-reducing Anaerobes, *E. Coli*, and *Staphylococci*. Besides, the initial C/N ratio has a no-significant effect on microorganism populations. Consequently, co-composting treatment has been demonstrated to be an effective and sustainable process for biowaste valorization and organic material sanitization. This study revealed that the co-composting technique could contribute effectively to transform poultry manures and industrial olive-oil by-products into a valuable resource. Aerobic heap co-composting made it possible to produce a sanitized and non-phytotoxic end-product that could be used as a soil organic amendment. This bioprocess has prevented contamination and reduced the density of all studied pathogenic microorganisms and improved the GI of all studied seeds (wheat, barley and lentil) leading to control of both sanitary and phytotoxic hazards associated with compost production from contaminated organic wastes. Additionally, achieving high thermal values during the bio-oxidative phase was revealed insufficient; it is also crucial to monitor the whole co-composting process to ensure a safe final compost with low pathogen contents leading to minimize the microbial hazards. This fact leads to the reduction of environmental issues due to the mismanagement of different biowastes composting and therefore contributes to the sustainability of agricultural practices. These findings could be completed by further studies focusing on composting process sanitation and contamination risk assessment, in order to contribute to a sustainable development policy capable to recycle and reuse agro-food by products.

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