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Inactivation of bacterial pathogenic load in compost against vermicompost of organic solid waste aiming to achieve sanitation goals: A review

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ABSTRACT

Waste management strategies for organic residues, such as composting and vermicomposting, have been implemented in some developed and developing countries to solve the problem of organic solid waste (OSW). Yet, these biological treatment technologies do not always result in good quality compost or vermicompost with regards to sanitation capacity owing to the presence of bacterial pathogenic substances in objectionable concentrations. The presence of pathogens in soil conditioners poses a potential health hazard and their occurrence is of particular significance in composts and/or vermicomposts produced from organic materials. Past and present researches demonstrated a high-degree of agreement that various pathogens survive after the composting of certain OSW but whether similar changes in bacterial pathogenic loads arise during vermitechnology has not been thoroughly elucidated. This review garners information regarding the status of various pathogenic bacteria which survived or diffused after the composting process compared to the status of these pathogens after the vermicomposting of OSW with the aim of achieving sanitation goals. This work is also indispensable for the specification of compost quality guidelines concerning pathogen loads which would be specific to treatment technology. It was hypothesized that vermicomposting process for OSW can be efficacious in sustaining the existence of pathogenic organisms most specifically; human pathogens under safety levels. In summary, earthworms can be regarded as a way of obliterating pathogenic bacteria from OSW in a manner equivalent to earthworm gut transit mechanism which classifies vermicomposting as a promising sanitation technique in comparison to composting processes.

Contents

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1. Introduction

In recent times, the rapid increase of organic residues has become a major problem in most developed and developing countries. It is necessary to explore effective waste disposal and/or management strategies. These enormous waste residues should be handled effectively by proficient methods in a sustainable and decentralized manner in order to alleviate adverse consequences on the environment and economy (Papargyropoulou et al., 2014; van der Werf et al., 2014; Pandey et al., 2016). The best option for the sustainable management of solid wastes is adopting the concept of resource recovery, reduction, reutilization, and recycling. Biological treatment methods such as composting (Munnoli and Bhosle, 2009; Purkayastha, 2012) and anaerobic digestion (Rounsefell et al., 2013; Quiroga et al., 2014) are implemented internationally to convert organic solid waste (OSW) such as food, yard and animal waste into useful soil amendment and biogas, respectively. Composting, among the different biological treatment methods, is perceived to be the most promising one since it is cheaper, tolerates Municipal Solid Waste (MSW) composition fluctuation and produces more stable organic fertilizers (Costa et al., 2016). Aerobic composting has recently received remarkable attention for recycling OSW (Chang and Chen, 2010; Zhou et al., 2014) because of its low-tech nature than anaerobic digestion, high organic content (Mohee and Soobhany, 2014), high nutritional capacity (Soobhany et al., 2015a) of MSW, profitable utilization of the finish product (Soobhany et al., 2015b) and less environmental damages than landfilling or incineration if properly conducted. After composting treatment of OSW, a valuable organic end product is obtained and is useful for agricultural purposes, which will benefit countries facing soil depletion (Hassen et al., 1998). The utilization of compost mitigates stresses on the environment; but it should be noted that plenty of zoonotic pathogens (fungi, virus, parasites such as helminth egg and bacteria) can be present in source-separated organic waste (Droffner et al., 1995; Petruzzelli, 1996; Strauch, 1996; Lalander et al., 2013; Hénault-Ethier et al., 2016) and can survive the composting process (Hassen et al., 2001). Alternatively, the application of raw or improperly composted organic manures on farms can induce the propagation and dispersion of pathogenic bacteria (Millner et al., 1994; Beffa et al., 1996) such as outbreak of *E. coli* (Beuchat, 2002; Jiang et al., 2002; Islam et al., 2005), *Salmonella* spp. and *Listeria monocytogenes* (Zhao et al., 1995) which delineated that a main challenge of composting is the uncertainty in bacterial pathogen inactivation. Pathogens inactivation after composting processes relates to the destruction of indicators organisms and is expected to occur if all particles of compost maintain temperatures greater than 55 °C for at least 3 days (US EPA, 1999; CCME, 2005).

In addition, Harrison (2004) observed problems regarding the quality of compost obtained from OSW in terms of sanitation although OSW compost is commonly believed to be a high quality product. In certain cases, the survivability of pathogenic organisms in a compost pile has been attributed to uneven heating temperatures (lower temperature on the surface of the piles), lack of homogenization owing to improper mixing (Gerba et al., 1995; Elving et al., 2010), cross contamination with working tools (Pereira-Neto et al., 1986; Harrison, 2004), leachate contact (Donaldson et al., 2013) and addition of feed materials or young compost to the mature compost. The regrowth and recontamination of pathogens in compost is widely reported, which are challenges of composting. Pandey et al. (2016) developed a heating system for the purpose of enhancing the temperature of compost pile for inactivating the pathogens in the compost but although *E. coli* persisted after the curing phase. The persistence of pathogenic bacteria after the curing phase is commonly termed as reactivation or regrowth. Regrowth is a problem only for certain bacterial pathogens such as *Salmonella* spp. and *E. coli* which, unlike some other bacterial species, viruses, protozoa and helminths, do not require a host organism for reproduction (Haug, 1993; Wichuk and McCartney, 2007). Storino et al. (2016) reported that composts obtained with meat waste showed the presence of *E. coli*, that exceeded the limits included in the technical purpose of European Commission (2014) for biowaste composted only in two out of twelve bins. The application of un-stabilized OSW, especially human waste, can cause health hazards such as rampant infections that have unbearable consequences (Sinha et al., 2010). Both environment concern and human health problems arise from successive accumulation of pathogenic organisms in human tissues, pathogenic bacteria uptake by vegetation and biomagnifications through the food chain. The verotoxins produced by *E. coli* can result in hemorrhagic colitis (diarrhea that becomes profuse and bloody), hemolytic uremic syndrome (bloody diarrhea followed by renal failure), and thrombocytopenic purpura (Pell, 1997). Death often occurs in patients with hemolytic uremic syndrome and thrombocytopenic purpura (Pell, 1997). The toxins produced by *Salmonella* spp. can result to clinical manifestations such as gastroenteritis, septicemia or typhoid fever (Ohl and Miller, 2001; Debbie, 2009). Strauch (1996) determined that *Salmonella*, *Shigella*, *E. coli*, *Enterobacter*, and Streptococci and the emergence of these bacteria from compost can harm compost trainers and agricultural laborers. In view with the increased interest on the public health, crop safety and environmental pollution, there is a growing demand for pathogen-free compost (Pandey et al., 2016). Additionally, pathogen-free compost can have unlimited use whereas lower quality compost may have restricted applications, for instance not in agriculture but for restoration of degraded sites or landfill capping. Actually, the subsistence of pathogenic loads in compost generated from OSW is a topic under consideration and the subject of massive debate which needs to be explored. In accordance with the last trend of environmental policies, vermicomposting, i.e. the treatment of organic wastes by earthworms acting in synergy with microbial populations, has proven to be an environmentally sustainable technology. Vermicomposting facilities have already been involved in domestic and industrial marketing in developed countries like Canada, United Kingdom, Spain, Italy, United States, Hong Kong, Australia, New Zealand, and Japan (Edwards et al., 2011). Yet, the decomposition of waste employing earthworms is growing popularity in developing nations owing to its low operational costs and high value vermicompost (Davison et al., 2006; Kumar and Shweta, 2011).

Many studies have been conducted on vermicomposting which discerned that a safe pathogen level can be achieved due to the microbial and enzymatic activity. It has been determined that vermicomposting may reduce pathogenic concentrations such as *E. coli*, *Salmonella enteriditis*, total and fecal coliforms, and helminthes ova in various categories of OSW (Monroy et al., 2009; Edwards, 2011). Nevertheless, the decline of the pathogenic indicators greatly depends on the earthworm species and/or the pathogen considered. However, one of the main issues associated to vermitechnology is the potential availability of high level human

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pathogens which could restrict the application of vermicompost as an organic fertilizer in agricultural systems. Edwards and Subler (2011) noted a research by Haimi and Huhta (1987) in which fecal *Streptococci* spp. augmented after vermicomposting. Moreover, Monroy et al. (2009) noticed an augmentation of total coliforms in vermicompost receiving high doses of pig slurry and their numbers were not systematically diminished by the addition of earthworms. Total coliforms may originate from soil surroundings as part of the native microflora (Byappanahalli and Fujioka, 1998), but fecal coliform are generally associated with animal waste (Smith, 2001; Contreras-Ramos et al., 2005), and this is why regulations are interested in fecal coliforms, but more often now in *E. coli*, as an indicator of potential animal feces contamination. Yet, only a few research works have dealt with human pathogens during the vermicomposting process to confirm if vermicomposting is indeed inefficient to control potential bacterial pathogens. Thus, these few researches raised doubt and hold opposing views concerning the effectiveness of vermicomposting in sanitization of OSW, partly because some conclusions appear contradictory. Owing to the innate drawback of the single processes, the combination of composting and vermicomposting together is progressively seeking interest for stabilization of different organic wastes most specifically with an increased pathogen reduction (Ndegwa and Thompson, 2001a; Nair et al., 2006; Lazcano et al., 2008; Hait and Tare, 2011a,b).

Although composting and vermicomposting are potentially useful methods to reduce or destroy pathogens, in some cases, the lack of a relationship between pathogen reduction and composting duration, temperature conditions, and possible mechanisms stemming from earthworm actions prompted a comparative literature review on the sanitation capacity of the two different composting systems. Despite a wealth of study on composting and vermicomposting, the mechanisms which are most important in pathogen inactivation during the vermicomposting process, and the process parameters which are necessary to ensure their effective reduction are not so well comprehended. This work is also indispensable for the specification of compost quality guidelines concerning pathogen loads which would be specific to treatment technology. In this regards, the key purpose of this review analysis was to perform a comparative assessment on the die-off of pathogenic load in compost against vermicompost of OSW aiming to achieve sanitation goals.

2. Biological characteristics of compost and vermicompost linking to human pathogens

Composting is an exothermic aerobic process which involves the transformation of organic wastes into relatively stable substances (Fialho et al., 2010). Composting allows the degradation of organic materials by microorganisms under controlled conditions, in which the organic material undergoes a thermophilic phase (45–65 °C) that enables sanitization of the waste by the inactivation of several pathogenic microorganisms (Lung et al., 2001; Mehta et al., 2014) due to the liberation of heat, carbon dioxide and water by microorganisms (Alidadi et al., 2005). Thus, composting is regarded as a cost-efficient biological management and stabilization technique for various OSW (Nasiru et al., 2013). According to Haug (1993), composting of OSW generates a product with significant reduction in pathogens compared to that in raw waste, reaching undetectable or nearly undetectable limits when properly conducted and monitored. Despite the large quantity of research which was in agreement with these guidelines; numerous studies have noted the subsistence of heat-resistant pathogens for composting systems (Inglis et al., 2010) even when the specified conditions are reached (Wichuk and McCartney, 2007). The explanations for the potential survivability of heat resistant pathogens might be due to a non-homogeneous heat distribution (Elving et al., 2010) and cross-contamination with working tools (Harrison, 2004). In contrast to composting, vermitechnology alone is a mesophilic process (<35 °C) to support earthworm life, where high temperatures are not usually desired for earthworm survival and thus, unable to slay pathogenic microorganisms. Vermicomposting is the processing of organic residues by earthworms which act in synergy with microbial populations for organic matter degradation. Although the precise mechanisms are not known, there is growing evidence that the vermicomposting process can lead to effective human pathogen elimination and sanitization of the processed material (Eastman et al., 2001). Moreover, an equal pathogenic load was obtained in mature compost and vermicompost produced using the same feedstock mix (Anastasi et al., 2005), implying that vermicomposting was as efficient as composting in achieving sanitization goals. On a great extent, the type of feed materials is a crucial feature in assessing the risk of human pathogen contamination with vermicompost. The bacterial communities of vermicompost are influenced by the variation of physico-chemical parameters during vermicomposting (Hénault-Ethier et al., 2015). However, the same health standards should be met for both thermophilic composts and vermicomposts with respect to acceptable pathogen loads. In certain cases and according to some researchers, preprocessing of substrates using traditional thermophilic composting for up to two weeks may be required to guarantee sufficient pathogen reduction (Ndegwa and Thompson, 2001a; Hait and Tare, 2011a,b).

3. Evolution of pathogens during composting

Composting urban waste reduces the volume of the waste, eliminates pathogenic organisms that may be present and limits the emission of unpleasant smelling compounds (Jakobsen, 1995). Dumontet et al. (2001) rated composting among the best available technologies for sanitizing and stabilizing biosolid's organic fractions. Stabilization and sanitization represent different concepts, for sanitization necessitates with the purpose of specific conditions be adhered sternly (de Bertoldi et al., 1988). In this context, the use of indicator pathogens for detection is reasonable. Table 1 summarizes publications which designated how pathogenic bacteria of OSW are affected by the composting process. It has been widely documented that composting could result in a decrease in *E. coli* below acceptable limit during composting of MSW (Hassen et al., 2001), broiler litter (Mohee et al., 2008), pig manure (Carthy et al., 2011), bovine manure (Millner et al., 2014) and co-composting of winery and distillery wastes (Bustamante et al., 2008). Enumeration of pathogen in green and food wastes compost was recently experiment by Pandey et al. (2016). Their research showed that *Salmonella* spp. declined to a significant extent (6.0–7.0 $\log_{10}$ CFU/g) in 5–34 days of composting of the green and food wastes. The sanitization of MSW through conventional composting was studied by Déportes et al. (1998) who noted a large decrease in fecal coliforms concentration notably from 5.2×10^7 CFU to less than 100, whilst *Salmonella* spp. decreased beneath detection limits (<1 MPN per 4 g dry solid). Similar obliteration in *Salmonella* spp. was obtained by other researchers (Vuorinen and Saharinen, 1999; Hassen et al., 2001; Krogmann et al., 2002; Carthy et al., 2011; Storino et al., 2016). Turner (2002) pointed out that the destruction of pathogenic compounds was affected by water content and nature of the substrate. However, although pathogen reduction or elimination especially *Salmonella* spp. by composting has been well documented (Vuorinen and Saharinen, 1999; Krogmann et al., 2002), composting regimes required to achieve elimination of pathogen indicators

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Table 1
Publications indicating how the pathogenic load is affected by composting (C).

Treatment	Duration of process (Days); maximum temperature attained	Parameter	Change in pathogenic load	Authors
C (manure)	NA	*E. coli*	↑	Hess et al. (2004)
C (chicken litter + peanut hulls; winter season)	57; >55 °C	*E. coli* *Salmonella* spp. *Listeria innocua*	↑ ↑ ↑	Erickson et al. (2010)
C (meat waste)	98; 67 °C	*E. coli* *Salmonella* spp.	↑ **ND**	Storino et al. (2016)
C (food waste)	70; 60 °C	*E. coli* *Salmonella* spp.	↑ **(95 %)** **ND**	Pandey et al. (2016)
C (municipal solid waste)	145; 65 °C	*E. coli* Total coliform Fecal Streptococci *Shigella* *Salmonella* spp.	↓ **(99%)** ↓ **(99%)** ↓ **(99%)** ↓ **ND**	Hassen et al. (2001)
C (winery and distillery wastes)	140; 60 °C	*E. coli* Total coliform Enterococci *Salmonella* spp. *Staphylococcus aureus*	↓ ↓ ↓ ↓ ↓	Bustamante et al. (2008)
C (broiler litter)	110; 66 °C	*E. coli* Total coliforms Fecal coliforms Fecal Enterococci *Salmonella* spp.	↓ **(82%)** ↓ **(70%)** ↓ **(80%)** ↓ **(60%)** **ND**	Mohee et al. (2008)
C (pig manure solids)	56; 66 °C	*E. coli* *Enterococcus* spp. *Samonella* spp.	↓ ↓ **ND**	Carthy et al. (2011)
C (bovine manure)	28; 70 °C	*E. coli* *Salmonella* spp.	↓ **(74 %)** ↓ **(85%)**	Millner et al. (2014)

Note: ↑ = signifies an augmentation in pathogenic load with % value in brackets; ↓ = signifies a reduction in pathogenic load with % value in brackets; ND = not detected (pathogen-free); NA = not available.

and pathogens vary widely principally due to the highly dynamic singularity of the composting system.

3.1. E. coli development

Adequate moisture content and efficient turnings during composting are the key factors to obtain a consistent and entire sanitization by elevated temperatures (Davis and Kendall, 2005). Conversely, some studies on OSW composting have demonstrated that an adequate elevated temperature for pathogen inactivation was hard to attain, entailing subsequent problems like sanitation (Bjorklund, 2002; Smith and Jasim, 2009; Niwagaba et al., 2009; Adhikari et al., 2012; Barrena et al., 2014). *E. coli* being the most representative bacterium in the group of fecal coliforms (Le Minor, 1984) is shown to survive (Droffner and Brinton, 1995; Christensen, 2002; Shepherd et al., 2007). In some earlier experiments, *E. coli* is shown to regrow in active compost at elevated temperatures (>50 °C) (Hess et al., 2004; Elving et al., 2010). Gagliardi and Karns (2000) and Bach et al. (2002) demonstrated the possibilities for *E. coli* to survive and move downwards to non-target environments through manure spreading. The reasoning for the survival of *E. coli* in composts was explained by the insufficiently self-heated areas of the piles (Elving et al., 2010), compost turning frequency (Harrison, 2004), short composting periods (Christensen, 2002). Hutchison et al. (2005) were unable to isolate *E. coli* O157:H7 in surface samples of livestock waste and bedding heaps composted for 8 days and attributed their negative results to the additional contribution of sunlight exposure. However, recovery and increase of the pathogens may very likely be due to incomplete inactivation associated to extreme dryness or low temperatures. In contrast, Harrison (2004) disagrees with the statement that high temperatures correlated with the survival of pathogenic microorganisms and suggests that the presence of *E. coli* during composting is a complex mix of concomitant factors and not simply the result of a thermal physical environment. Thus, such facts highlight the priorities in searching for efficient treatment processes with a few elementary modifications to give a more extensive zone for high temperature.

3.2. Salmonella spp. development

Salmonella is a zoonotic bacterium of high concern in farming because it is infectious to animals and can stay alive for a long time in the soil (Jamieson et al., 2002). Despite the large body of work which has been conducted on composting, numerous works have determined presence of bacterial pathogen especially *Salmonella* spp. during composting of different organic matter even though the time-temperature criteria were attained (Pourcher et al., 2005; Wichuk and McCartney, 2007; Grewal et al., 2007; Bustamante et al., 2008; Millner et al., 2014). For instance, *Salmonella* spp. has been shown to regrow or replicate in mature thermophilic compost (Sidhu et al., 2001; Erickson et al., 2010), recontaminate windrows (Brinton and Droffner, 1995) even if the time–temperature criteria were met. The reason for the regrowth incongruity might be due to the temperatures that are not homogeneous into the whole composting heap (Gerba et al., 1995); and recontamination occurs through turning of the substance (Pereira-Neto et al., 1986). The key source for *Salmonella* spp. is mainly animal manure (Létourneau et al., 2010), food wastes; mostly from unpasteurized orange juice, contaminated tomatoes (Debbie, 2009) meats, poultry, milk and its derivatives (Hassen et al., 2001). According to Brinton and Droffner (1995), some mutant

strains of *Salmonella* spp. may withstand high temperatures (42–54 °C). The occurrence of *Salmonella* spp. in compost is considered as a major problem in regards with hygienic quality (Yanko, 1995; Brinton and Droffner, 1994) since these bacteria are ubiquitous and have a capacity for very fast growth (Hassen et al., 2001). Thus, composting practices does not always guarantee an absolute sanitization of the end-product although relatively high thermal values were achieved. The ability for the growth of *Salmonella* spp. can be influenced by other factors notably, moisture content, nutrient availability or viable microbiota (Bustamante et al., 2008). In order to ensure an effective composting process for the production of a sanitized end-product, it is primordial to supervise the process and particularly the curing phase since a microbial reactivation is possible in this stage.

3.3. Other pathogens development

A great deal of research documented pathogens survivability during composting (Droffner et al., 1995; Nair et al., 2006) which pointed out that thermophilic composting conditions should not be regarded as the only safe sanitation method. It was found that composting technology could provide a decrease in total coliform as researched by Bustamante et al. (2008) but a regrowth of total coliform could also arise during composting and this regrowth has been hitherto described by other authors (Hassen et al., 2001). This secondary growth of coliform was due to the recontamination during the turnings of the windrows (Hachicha et al., 1992), large diversity of indigenous microbes (Nair et al., 2006) and dependency on the storage conditions (Erickson et al., 2010). Inglis et al. (2010) found the persistence of Campylobacter in buffalo waste compost and this survivability might be due to heat resistant mutants. The pathogenic load decay is one of the main factors during compost storage (Gale, 2004) or application to land of the composted matter for the evaluation of human health risks.

4. Evolution of pathogenic organisms during vermicomposting

It has been previously reported that the thermophilic phase during the composting process reduces and/or inactivates pathogenic groups. Nevertheless, it has been generally accepted that human pathogens are also destroyed during vermicomposting (Hait and Tare, 2011a,b; Yadav et al., 2011) most likely by way of an antagonism mechanism. This theory has also been explained by Monroy et al. (2009) who elaborated that the contacts among earthworms and the microbial community decreased some bacterial pathogens indicators. Even though a few studies have been conducted on pathogens concentration in vermicomposting, the majority of them support the potential of vermicomposting to consistently and largely diminish pathogens from contaminated substrates. Moreover, a more rapid and complete removal of pathogen has been achieved in vermicomposting using high densities of earthworms than thermophilic composting of the same materials (Eastman et al., 2001). This full elimination of pathogens was due to the addition of earthworms in the vermireactor (Yadav et al., 2012) which is a focal aspect of pathogenic materials during vermiprocessing. Table 2 showed some publications which indicated how potentially pathogenic bacteria of OSW are affected by vermicomposting together with the different earthworms species employed.

4.1. E. coli progression

Numerous researches revealed the capability of vermicomposting to effectively eliminate *E. coli* (Bajsa et al., 2005; Mainoo et al., 2009; Kumar and Shweta, 2011; Aira et al., 2011) in different type of OSW. The decrease or complete eradication in *E. coli* after vermicomposting of OSW could be explained by a constant antimicrobial response on gram-negative bacteria from the gizzard through the intestinal tract of the earthworms as identified by Finola et al. (1995). Hénault-Ethier et al. (2016) observed that *E. fetida* negatively influenced *E. coli* survival and detected an earthworm density independent relationship (survival of *E. coli* did not decrease with increasing earthworm density). Nevertheless, their results disagree with Williams et al. (2006) who noticed that earthworms (*L. terrestris* and *D. venata*) broaden the survival of *E. coli* in compost due to earthworm predation on protozoan that nourish on *E. coli* (Bonkowski and Schaefer, 1997; Brown and Doube, 2004). Hénault-Ethier et al. (2016) discerned that certain nutrients (i.e. labile sugars abundance, but not labile C) could have contributed indirectly in *E. coli* survival. In this series of experiments, *E. coli* abundance decline in source-separated organic waste could conceivably be linked to antagonisms with bacteria in the vermicompost, as antagonistic bacterial genus or species were isolated from vermicompost, and *E. coli* declined more rapidly in fresh as opposed to autoclaved vermicompost. This confirms the assumption made by other authors (Eastman et al., 2001; Sidhu et al., 2001; Panikkar et al., 2004) who hypothesized that antagonistic populations may allow pathogens survival on longer time periods and also agrees with the suggestion that *E. coli* may be less competent competitors for labile C sources than the original microbial flora.

4.2. Salmonella spp. progression

Vermicomposting potential for OSW has been addressed as beneficial human pathogen stabilization in terms of *Salmonella* spp. Several authors observed a decline in populations of pathogenic *Salmonella* spp. during vermicomposting of sewage sludge by *E. fetida* (Mitchell, 1978; Cardoso, 2002; Bajsa et al., 2005; Rodriguez-Canché et al., 2010), human waste by *E. fetida* (Lalander et al., 2013), pineapple wastes by *E. eugeniae* (Mainoo et al., 2009), biosolides by *E. fetida* (Eastman et al., 2001; Contreras-Ramos et al., 2005) and other organic wastes (Kumar and Shweta, 2011). It was found in an earlier study made by Brown and Mitchell (1981) that *E. fetida* decreased *Salmonella* spp. by 97.8–99.9% compared to cultures with no earthworms. Also, Contreras-Ramos et al. (2005) found that vermicompost obtained from biosolides mixed with other amendments contained fewer than 3 CFU per 4 g *Salmonella* spp. To emphasize that interaction with antagonistic microorganisms is important compared to high temperatures alone, the suppression of *Salmonella* is actually more efficient at 55 °C than at 70 °C (Ryckeboer et al., 2003).

4.3. Total coliforms progression

It is known that earthworms greatly reduced total coliform bacteria during vermicomposting. The passage through the guts of the earthworm reduced the populations of total coliforms by 98% relative to that in fresh pig slurry, only in the low dose vermireactors, implying a density reliant effect of earthworms on these pathogens (Monroy et al., 2008, 2009). The diminution in total coliforms was due to earthworm digestive processes that constitute fine grinding of cells (Monroy et al., 2008, 2009; Edwards, 2011) and viable relations between coliforms and microorganisms which are particular to the earthworm gut microflora (Brown and Mitchell, 1981; Horn et al., 2005). Their results supported the hypothesis that the diminution in coliforms from the inoculation of earthworms in the low dose vermireactors was completely documented by the gut transit effect since there was a straight connection between the intake of pig slurry by *E. fetida* and the drop off in total coliforms. The decline in total coliforms during vermicomposting

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Table 2
Publications indicating how the pathogenic load is affected by vermicomposting (V) and pre-composting followed by vermicomposting (C + V).

Treatment	Duration of process (Days); maximum temperature attained	Earthworm species used	Parameter	Change in pathogenic load	Authors
V (pig slurry in high dose vermireactors)	60; NA	*E. fetida*	Total coliform	↑	Monroy et al. (2009)
V (human waste)	750; NA	*E. fetida*	*Salmonella* spp. *Enterococcus* Coliforms Coliphages	↓ ↓ **(50 %)** ↓ **(30 %)** ↓ **(60 %)**	Lalander et al. (2013)
V (biosolides)	158; NA	*E. fetida*	Fecal coliforms *Salmonella* spp. Enteric viruses Helminth ova	↓ **(64%)** ↓ **(86%)** ↓ **(46%)** ↓ **(19%**	Eastman et al. (2001)
V (biosolides)	60;NA	*E. fetida*	*Salmonella* spp. Fecal coliforms *Shigella* spp. Helminth ova	↓ **ND** **ND** **ND**	Contreras-Ramos et al. (2005)
V (pig slurry in low dose vermireactors)	60; NA	*E. fetida*	Total coliform	↓ **(98%)**	Monroy et al. (2009)
V (pineapple wastes)	140; NA	*E. eugeniae*	*E. coli* *Salmonella* spp. *Aspergillus*	↓ **(31–70%)** ↓ **(31–70%)** ↓ **(78–88%)**	Mainoo et al. (2009)
V (sludge)	60; 36 °C	*E. fetida*	*Salmonella* spp. Fecal coliforms	↓ ↓	Rodriguez-Canché et al. (2010)
V (sewage sludge)	84; NA	*E. fetida* + *P. excavatus* + *E. eugeniae*	Coliforms *E. coli*	**ND** **ND**	Sinha et al. (2010)
V (cow manure)	21; NA	*E. andrei*	*E. coli* Fecal coliforms Fecal enterococci	↓ ↓ ↓	Aira et al. (2011)
V (faeces)	NA; <35 °C	NA	*E. coli*	↓	Hill and Baldwin (2012)
V (source separated organic waste)	81; NA	*E. fetida*	*E. coli*	↓	Hénault-Ethier et al. (2016)
C + V (kitchen waste)	21; 60 °C	*L. rubellus* + *E. fetida*	*E. coli* *E. faecalis* *Salmonella* spp.	↓ **ND** **ND**	Nair et al. (2006)
C + V (dairy manure + paper waste)	84; 30 °C	*E. fetida*	*E. coli*	**ND**	Mupondi et al. (2010)
C + V (human fecal slurry)	180; 35 °C	*E. fetida*	Total coli Fecal coli *Salmonella* spp. Helminth ova	**ND** **ND** **ND** **ND**	Yadav et al. (2012)

Note: ↑ = signifies an augmentation in pathogenic load; ↓ = signifies a reduction in pathogenic load with % value in brackets; ND = not detected (pathogen-free); NA = not available.

were similar to those reported by other authors (Murry and Hinckley, 1992; Eastman et al., 2001) during stabilization other organic waste though coliforms may not be sufficiently reduced for use of the end product without an additional sanitation step. Yadav et al. (2010) found an absolute elimination in total coliform (>8 log diminution) in large 60 kg batch experiments. Also, Monroy et al. (2008) discerned that the decline in total coliform numbers varied among four earthworm species (*E. fetida*, *E. andrei*, *L. rubellus* and *E. eugeniae*). However, on the contrary, continual existence of total coliforms was higher in the high dose vermireactors and their populations were not decreased by the addition of earthworms (Monroy et al., 2009). A possible explanation to the apparent

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discrepancy in survivability might be due to variability in aeration in the vessels.

5. Evolution of pathogens during integrated composting-vermicomposting system

Due to the inherent limitations of the individual processes, the integration of composting and vermicomposting together is increasingly receiving attention as a result of attaining stabilized end product (Tognetti et al., 2007) and to a large extent, as an efficient approach for organic waste sanitation (Frederickson et al., 1997). In the past few years, some research works have attempted to study stabilization of various type of OSW through integration of composting and vermicomposting (Alidadi et al., 2005; Nair et al., 2006; Lazcano et al., 2008; Mupondi et al., 2010; Hait and Tare, 2011a,b; Yadav et al., 2012; Soobhany et al., 2015a–c). Sanitization of OSW (Ndegwa and Thompson, 2001a) and reduction of toxic heavy metals are enabled by an integrated composting-vermicomposting system (Garg and Gupta, 2011; Fornes et al., 2012; Wang et al., 2013; Song et al., 2014; Soobhany et al., 2014; Soobhany et al., 2015b,c; Bakar et al., 2015; He et al., 2016). The sequential combination of composting and vermicomposting also results in a very much fine structure of the end product (vermicompost) with homogeneity in particle size distribution (Atiyeh et al., 2000; Ndegwa and Thompson, 2001a) and in an increase in bioavailability of nutrients content (Soobhany et al., 2015a; Swarnam et al., 2016). Also, inoculation of earthworms to the material after the thermophilic phase of composting renders the treatment more cost-effective since vermicomposts have a higher market value with a selling price of $50–$1000 (U.S) per ton (Edwards et al., 2011) compared to composts and environmentally productive waste management strategy (Sim and Wu, 2010). Moreover, the earthworms worked well after the heat of the initial decomposition (Logsdon, 1994) and gave successful outcomes in accelerating the process and thus shortening the time for curing (Ndegwa and Thompson, 2001b). Ndegwa and Thompson (2001a) found that simple composting followed by vermicomposting was more effective in reducing pathogen numbers than simple composting alone. For example, *E. coli* was greatly reduced and *E. faecalis* was fully eliminated following vermicomposting, than after thermophilic composting alone whilst materials subjected to thermophilic composting only contained higher levels of pathogens (Nair et al., 2006). Mupondi et al. (2010) noted a full elimination of *E. coli* from the final end product owing to the combined thermophilic composting and vermicomposting whereas vermicomposting only managed to reduce the pathogen population. Additionally, *Salmonella* spp. was removed completely when combining composting and vermicomposting processes of organic waste (Nair et al., 2006; Yadav et al., 2012). However, in spite of various studies on vermicomposting technology, very little information has so far been available on the inactivation of pathogenic bacteria in OSW since most of the integrated composting-vermicomposting experiments focus on metal toxicity. Thus, the integrated composting-vermicomposting process for OSW treatment obviously necessitates further experimental studies with arrays of reinforcing the hypothesis that preceding vermicomposting with a thermophilic pre-composting step could be the most favorable process to assure the inactivation of pathogenic bacteria in vermicomposts.

6. Effects of possible mechanisms on earthworms in response to reduction in pathogens

Although a number of human pathogenic microorganisms are shown to reduce during the vermicomposting of a variety of OSW (Eastman et al., 2001; Contreras-Ramos et al., 2005; Craig and Ankers, 2006), few information is available concerning the mechanisms involved which influence the intensity of this effect. Addressing the bacterial pathogenic communities during vermicomposting is vital for a better understanding since earlier studies have found that bacteria being natural decomposers of OSW are abundant in vermicomposting (Rousk et al., 2010). However, the majority of previous investigations on vermicomposting focused exclusively on bacterial characteristic (Sen and Chandra, 2009; Vivas et al., 2009; Yasir et al., 2009; Fernandez-Gomez et al., 2012), studies on the possible mechanisms of earthworms on pathogen reduction are scarce. According to Monroy et al. (2009), a potential mechanism for pathogen reduction in vermicompost is the short-term indirect effects of the earthworms on bacterial communities. The digestive tract of earthworms contained a specific microbial group which is thereby aided by fatty acid decomposition of gut microorganisms from *L. terrestris* (Sampedro et al., 2006; Sampedro and Whalen, 2007) and these gut microorganisms may outperform consumed microbes because of enhanced abilities in the gut medium (Byzov et al., 2007). In cases where the population of earthworms is reared at high densities in vermicomposting systems (Edwards and Bohlen, 1996), the gut and the cast associated processes are the chief factors involved in identifying the trait of the microbial biomass (Domínguez, 2004) with no change in microbial community or decomposer activity (Aira et al., 2007). Specific interactions between earthworms and microorganisms rely on the microbial group considered (Schönholzer et al., 1999; Brown and Doube, 2004; Byzov et al., 2007) and their activity alters greatly upon passage through the pharynx of earthworms (Pedersen and Hendriksen, 1993; Karsten and Drake, 1995). Earthworms render the environment more unsuitable for pathogens (Contreras-Ramos et al., 2005; Kadam et al., 2008) and produce antibiotics which kill the pathogenic organisms (Sinha et al., 2010). The fate of bacteria pathogens during gut transit is variable (Wolter and Scheu, 1999; Aira et al., 2002) depending on pathogens considered or earthworm species, bacterial numbers, metabolic state, substrate and feed type (Brown and Doube, 2004; Pedersen and Hendriksen, 1993).

It is obvious that the effect of earthworms and the vermicomposting system on human pathogens can be somewhat complex. In vermicomposting, killing of pathogens is prominently achieved through earthworm actions such as intestinal action, suppressive activity of gut fluids and selective grazing (Domínguez and Edwards, 1997) and release of secretion of coelomic fluids that have antibacterial properties (Sinha et al., 2002, 2010; Kumar and Shweta, 2011). Brown and Mitchell (1981) also provided solid evidence that the stimulation of endemic bacteria by earthworm activity may lead to pathogen destruction through competitive or antagonistic interactions. The mechanisms by which pathogens might be decreased or eliminated consist of the straight influences of mechanical interruption owing to intake and crushing action of the earthworms' gizzard and diffusion of microorganisms by enzymatic digestion and absorption (Edwards and Subler, 2011). The metabolic quotient or specific activity of the microbial biomass (qCO_2; microbial respiration per unit biomass) can be used as a measure of microbial efficiency to earthworm activity; higher values of qCO_2 indicate that microbial communities are under conditions of higher stress (Edwards et al., 2011). It has been previously researched by Edwards et al. (2011) that the activity of earthworms reduced the metabolic quotient after vermicomposting for a period of one month, indicating that microbial communities used the available energy more efficiently in the presence of earthworms. Though past studies have mentioned alterations of microbial community and changes in physico-chemistry of the substrate over time (Hénault-Ethier et al., 2015) during vermicomposting, the mechanisms engrossed are still ambiguous which require profound researches for elucidating a promising approach.

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Table 3
Compost quality assessment in terms of pathogen concentration and phytotoxicity in different European countries.

Country	Regulation	Application	Parameter	Limit value
Austria	Statutory Guideline	Land reclam.	*Salmonella*	Absent
		Agriculture	*Salmonella*	Absent
			E. coli	If positive, recommendation for use
		Sacked, sport/playground	*Salmonella*	Absent
			E. coli	Absent
			Camylobacter	Absent
			Listeria	Absent
		Technical use	No requirements	–
		Horticulture	Weeds/propagules	Germination ≤3 plants/L
Belgium	VLACO		Process control	Time, temp relation
			Weeds	Absent
			Salmonella	Absent
Czech Rep.	Biowaste Ordinance		*E. coli*	$<10^3$ CFU/g
			Enterococcae	$<10^3$ CFU/g
			Salmonella	Absent
Denmark	Biowaste Ordinance	Controlled sanitized compost	*E. coli*	<100 CFU/g FM
			Enterococcae	<100 CFU/g FM
			Salmonella	Absent in 25 g
France		Gardening/retailer	*Salmonella* spp.	Absent in 1 g
			Helminth ova	Absent in 1 g
Germany	Biowaste Ordinance		Tomato seeds[a]	Germination rate/sample: ≤2%
			Salmonella senft.[b]	Absent in 50 g sample
			Weeds/propagules	Germination ≤2 plants/L
			Salmonella	Absent in 25 g
Italy	Fertilizer law		*E. coli*	≤1000 CFU/g
			Weeds	Germination index ≥60%
			Salmonella	Absent in 25 g
Netherlands	Beoordeli ngsrichtlijn keurcompost	All application	Plasmodoph brass	Absent
			Weeds	Germination ≤2 plants/L
			Ascaris	Absent
			Trichuris	Absent
Slovenia	Decree on the treatment of biodegradable was		*Salmonella* spp.	Absent in 25 g
Spain			*Salmonella* spp.	Absent in 25 g
			E. coli	<1000 MPN/g
United Kingdom	PAS 100 voluntary standard	All application	*Salmonella* spp.	Absent in 25 g
			E. coli	≤1000 CFU/g
			Weeds/propagules	Germination weed plant: 0/L

Adapted from Saveyn and Eder (2014).
[a] Process validation.
[b] Compost production.

7. Global compost quality assessment in terms of pathogen concentration

Vermicomposting system of OSW is a recycling strategy which could result in a closer population's involvement to achieve progress objectives for hygiene, health and employment (United Nations, 2006). The main bottleneck for the wider application of upcycling technologies such as vermicomposting is the absence of a thermal sanitation phase, which would make it a recognized system to significantly or further reduce pathogens (CCME, 2005; US EPA, 1994). When employing a non-standard protocol, the best complete pathogen evaluation should be conducted where a passing grade would have no viable *Ascaris* spp. (<0.5 viable ova/g ds), median of all samples <1 MPN per 4 g ds for *Salmonella*, and median of all samples <10 MPN fecal coliform/g ds, not more than 20% >1000 MPN/g ds, and none >10,000 MPN/g (Haug, 1993). Regarding pathogen elimination during composting, some guidelines have been established with the major aspects requiring 55 °C for at least 3 days (US EPA, 1999; CCME, 2005), 53 °C for 5 days, 55 °C for 2 days and 70 °C for 30 min for destruction of pathogens (US EPA, 1992) or the final composts should hold $<\log_{10}3$ cells/g dry wt of fecal coliforms (CCME, 1996). For vermicomposts generated from vermicomposting to be regarded as an effective treatment for Class A pathogen stabilization, the end product need to have a three to fourfold decrease (defined as being divided into a specified number of parts) of pathogen indicators (US EPA, 1994). Thus, these rules could give a proper stabilization of OSW to guarantee public health concern with the vermicomposted product. Though, if compost generated from manure and other manure byproducts are to be sold commercially, some microbiological criteria should be followed so as to lessen threats to manufacturers, handlers and users. For the marketing of compost generated from animal wastes, it must abide with EU animal by-product rules (EC/1774/2002 amended by EC/208/2006; EC/1069/2009).

It was found earlier that phytotoxic intermediates and synthetic compounds could not be degraded fully in an incomplete composting system (Getahun et al., 2012). Though the agronomic value (% of organic carbon) and toxic heavy metals content are generally well established in compost quality regulations, a deficiency in consistency can be seen for direct methods applied to assess the detection of pathogens, as depicted in Table 3, where regulation or guidelines established in different countries are listed. For the most part, detection of *Salmonella* and *E. coli* are the frequent criteria with reference to pathogen survivability and germination index for phytotoxicity. In several European Member States, regulations also endow with techniques to obviate pathogens and phytotoxicity in

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compost through the need of a specific temperature profile during the stabilization process. The detection of *Salmonella* spp. is required by all states in Table 3 (except Netherlands) and estimation of *Salmonella* along with *E. coli* is required to substantiate the presence of pathogens in compost. Belgium VLACO relies on process control while the Netherlands consider different parasitic nematode worms' species such as Plasmodoph brass, *Ascaris* and *Trichuris*. It seems remarkable to highlight that the evaluation of phytotoxicity by means of germination index is enforced in some Member States such as Austria, Italy, Germany, the Netherlands and the United Kingdom: in Italy and Germany, it is a parameter imposed by law whereas, in the other countries, an investigative maximum value is given in present guidelines.

Though the control of the input of feedstock and proper management of the composting process are important, the end product should always be assessed in terms of stability, maturity and quality criteria in order to safely use compost for agricultural purposes. Potentially contaminated compounds that are ubiquitous in the environment can also be present in OSW going to composting. Thus, it is essential to quantify the potential for uptake of toxins and/or pathogens into the food chain, resulting from the use of waste produced composts within the supply chain (Hough et al., 2012). Compost stability is related to the level of activity of the microbial biomass (Cesaro et al., 2015). In contrast, compost maturity refers to the degree of humification and implies the absence of both phytotoxic compounds and pathogens (Bernal et al., 1997). Maturity is partially affected by the relative stability of the material, since phytotoxic compounds are produced by the microorganisms in unstable composts (Bernal et al., 2009). However, the insertion of stability criteria for compost quality assessment is a significant issue since a stability condition can avoid the introduction of contaminated materials into soil. Stability is an essential aspect of composting in relation to its field application and pathogen regrowth (Edwards et al., 2011). Yet, it could be inferred that different feedstock characteristics influences compost stability thereby the sanitation capacity of the final compost.

8. Conclusions

From a hygienic and health viewpoint, composting of OSW could slightly increase the presence of *E. coli* levels in the final compost owing to the presence of heat resistant mutants. Unlike for OSW vermicompost, *Salmonella* spp. has been shown to regrow or replicate in mature thermophilic compost and the reason for the regrowth incongruity might be due to lack of homogenization owing to improper mixing in the composting heap. Still, composting could be a sustainable strategy for OSW management with a low level of pathogens, provided that the system should be controlled effectively by a thorough handling and proper management of the composter vessels. During vermicomposting, the passage of organic residues through the gut of certain earthworm species can cause die-off of some pathogenic indicators (*E. faecalis, E. coli, Salmonella* spp., total coliform) and this process is perhaps the focal factor entailed in the decline or elimination in certain pathogens. Earthworms can be regarded as a way of obliterating pathogenic bacteria from OSW owing to its digestive processes and gut way effect which classifies vermicomposting as a promising sanitation technique in comparison to conventional composting processes. In addition, some studies suggest that the interactions between microorganisms in vermicomposting are also defined as potential pathogen mechanism eradication and may have a greater effect than the earthworms themselves in the reduction of pathogenic indicators. However, the integrated composting-vermicomposting process for OSW treatment obviously warrants further studies with arrays of reinforcing the hypothesis that preceding vermicomposting with a thermophilic pre-composting step could be the optimal process to guarantee the minimal survival of pathogens in vermicomposts.

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