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ORIGINAL ARTICLE

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Impacts of different treatment methods for cattle manure on the spread of faecal indicator organisms from soil to lettuce in Nigeria

Vince A. Chukwu[1] | Jo U. Smith[1] | Norval J. C. Strachan[1] | Lisa M. Avery[2] | Smart O. Obiekezie[3]

[1]Environmental Sciences, School of Biological Sciences, University of Aberdeen, Aberdeen, UK

[2]James Hutton Institute, Aberdeen, UK

[3]Department of Microbiology, Nasarawa State University, Keffi, Nigeria

Correspondence
Vince A. Chukwu, Environmental Sciences, School of Biological Sciences, University of Aberdeen, 23 St. Machar Drive, Aberdeen AB24 3UU, UK.
Email: v.chukwu.16@abdn.ac.uk

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Abstract

Aim: This study investigated impacts of different organic waste treatment methods on reduction and spread of faecal indicator organisms to food crops in a developing country.
Methods and Results: Fresh cattle manure was subjected to three different treatments; anaerobic digestion, burning and composting. *Escherichia coli*, coliforms and nitrogen content of cattle manure were measured before and after treatment in the amended soil and harvested lettuce. All treatments significantly reduced *E. coli* and coliform counts but differed in the ratio of *E. coli* or coliforms to nitrogen. Application of the recommended nitrogen dose of 120 kg ha^{-1} as bioslurry resulted in significantly lower *E. coli* and coliform contamination of soil than the same nitrogen rate applied as compost or ash. The *E. coli* content of lettuces grown on soil amended with treated wastes at recommended rates did not differ between treatments but was significantly lower than in lettuces grown on soil amended with untreated manure.
Conclusions: Treatment of manure before use as an organic fertilizer significantly reduces potential contamination of both soil and food crops with *E. coli* and coliforms. To best reduce the spread of *E. coli* from organic fertilizers, manures should be treated by anaerobic digestion.
Significance and Impact of the Study: Information from this study quantifies potential risks associated with use of manures in growing food crops by determining the ratio between pathogen content and required nitrogen application rate.

KEYWORDS
anaerobic digestion, cattle manure, coliforms, composting, *Escherichia coli*, lettuce, nitrogen

INTRODUCTION

Use of organic wastes as fertilizers is on the rise globally and is attracting attention of the food production sector (Maffei et al., 2016). This serves as an alternative means of disposing organic agricultural wastes thereby reducing pollution (Sun et al., 2012). However, use of organic wastes as amendments to agricultural soil has potential to increase spread of pathogens to food crops and the wider environment (Elving, 2009).

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Use of organic fertilizers in developing countries

In developing countries, organic fertilizers are mainly sourced from agricultural wastes (such as cow manure) or municipal solid wastes (Oyesola & Obabire, 2011). Poultry and goat manures have also been reported to be widely used (Adhikari et al., 2016). Treated organic fertilizers, such as bioslurry, obtained as a by-product of biogas production, are also applied. These wastes serve as valuable sources of nutrients, nitrogen (N), potassium (K) and phosphorus (P), while also increasing the organic matter content of the soil (Roy et al., 2010; Shabani et al., 2011; Yadav et al., 2017). This has been demonstrated to improve soil structure, so reducing bulk density and increasing water holding capacity and plant available water (Ramos, 2017). Crop yields have been widely observed to be improved with application of organic wastes (e.g. Adhikari and Subedi, 2016). Significant reduction in losses of soil by erosion due to application of organic fertilizers has also been demonstrated (e.g. Angin and Yaganoglu, 2011).

Pathogens from organic wastes in developing countries

Although of benefit to crop production and soil stability, organic wastes may contain pathogenic micro-organisms, such as bacteria, viruses, parasites and pathogenic fungi (Elving, 2009), and so can present potential risks to both human, animal and crop health, especially if applied in an untreated form. These pathogenic micro-organisms can be transmitted to food crops by splashing contaminated soil particles from the surface of the soil to the crops during rainfall or overhead irrigation. Direct contact of plant surfaces with animal manure can contribute to contamination of food crops (Alegbeleye et al., 2018). Transmission of pathogens to food crops has also been demonstrated to occur through attachment to plant surfaces as well as by uptake through plant roots (Burris et al., 2020).

A number of studies in developing countries have identified common pathogens in foods associated with organic amendments (Atidégla et al., 2016; Ceuppens et al., 2014; Lennox et al., 2015). In Nigeria, bacterial organisms, *Listeria monocytogenes*, *Escherichia coli* O157:H7 and *Salmonella enterica*, are the most common pathogens associated with contamination of food crops grown with organic fertilizers (Chang et al., 2013; Nair et al., 2015; Reuben & Makut, 2014). Pathogenic fungi, *Aspergillus fumigatus*, *Trichoderma* spp., *Aspergillus niger*, *Rhizopus* spp., *Penicillium* and *Fusarium* spp., have also been detected in fresh vegetables grown with organic fertilizers in Nigeria (Van de Perre et al., 2014). These pathogens have been shown to survive for longer periods in manure-amended than unamended soils (Çekiç et al., 2017; Franz et al., 2008). Therefore, it may be beneficial to reduce the number of pathogens in organic wastes before application.

Household treatment of organic wastes in Nigeria

Physical methods of sanitizing organic wastes include heating and burning (Smith et al., 2015). Biological methods include composting and anaerobic digestion (Maffei et al., 2016).

Burning

Burning involves thermal treatment in the presence of oxygen at temperatures of up to 850℃. Wastes are converted to carbon dioxide, water and a noncombustible solid ash residue, which is available to apply to soils. This is one of the most commonly used methods in Nigeria to dispose of organic wastes and reduce the pathogen content (Chukwuemeka et al., 2012). Turner et al. (2000) showed that treatment of organic wastes by burning has the potential to significantly reduce the pathogens contained in pig slurry. In a laboratory study, several pig viruses present in pig slurry supplied by the veterinary laboratory agency (United Kingdom) were deactivated within 3 min by heating the slurry; for foot and mouth virus, heating at 67℃ was required, for Aujeszky's disease, 63℃, and for classical swine fever, 62℃. Upscaling to a pilot treatment plant, foot and mouth disease virus was inactivated at 66 or 61℃ in hot water or slurry respectively; Audjeszky virus at 61℃ in hot water or slurry and the classical swine fever viruses were inactivated at 62℃ in hot water and 50℃ in slurry.

Composting

Composting involves the microbial degradation and stabilization of organic wastes in the presence of oxygen (Stentiford & de Bertoldi, 2010). It is an effective means of reducing the content of zoonotic organisms, such as vero-toxigenic *E. coli* and *Salmonella* spp. (Gurtler et al., 2018). The use of compost serves as a cheap, alternative source of nutrients for agricultural use in Nigeria, with the potential of increasing agriculture production by improving soil fertility. However, composting in this region is not as widely practiced as in Europe and Asia (Olukanni & Aremu, 2017). Composting provides rapid, controlled biological oxidation of organic wastes by micro-organisms (active, heat generating stage) and results in an odourless product with reduced volume and weight (Font-Palma, 2019). Reduction of the pathogenic content occurs during the maturation stage (Avery et al., 2014).

The co-composting of organic wastes to generate organic fertilizers used in growing cabbage and lettuce crops resulted in significantly lower microbial loads of coliforms, *E. coli*, *Salmonella* spp., *Shigella* spp. and *Klebsiella* spp. (mean value of microbial loads = 1.18×10^7 CFU per ml) on harvested vegetables than even chemical fertilizers (2.45×10^9 CFU per ml) or the control plot with no treatment (5.2×10^8 CFU per ml) (Torgbo et al., 2018). Soobhany (2018) observed that composting organic municipal solid waste led to significant reduction in total coliforms (from 3.13–3.57 to 1.52–2.89 $\log_{10}$ most probable number [MPN] per gram) and *E. coli* counts (from 6.00–6.15 to 4.72–4.96 $\log_{10}$ MPN per gram). *Salmonella* spp. free organic fertilizer suitable for application as agricultural amendments was also generated from the composting of the organic wastes (Soobhany, 2018).

Anaerobic digestion

Anaerobic digestion involves degradation of organic matter by different groups of micro-organisms in the absence of oxygen (Font-Palma, 2019). Anaerobic digestion is widely used globally because of its potential to produce biogas, an alternative source of household energy (Font-Palma, 2019). However, in Nigeria, anaerobic digestion of food wastes and animal manure is not a common practice (Akpan et al., 2019). This could be due to lack of awareness and appropriate configuration of the treatment system (Akpan et al., 2019). In other sub-Saharan African countries, such as Ethiopia, there is growing interest in the use of anaerobic digestion to reduce spread of faecal indicator organisms (FIOs) to the wider environment. In Ethiopia, Nakamya et al. (2020) demonstrated that anaerobic digestion of cattle manure resulted in significantly lower counts of FIOs in both the inside and outside environment of households with biogas digesters installed. Significant reductions in *Bacillus* spp., *Clostridium* spp., *Klebsiella* spp., *E. coli*, *Aspegillus* and *Shigella* spp. by anaerobic digestion at ambient temperatures have been demonstrated by Alfa et al. (2014). They observed that anaerobic digestion of fresh animal waste for 30 days at an average ambient temperature of 30 ± 2℃ and pH between 6.5 and 8.0 resulted in a 90% and 75% reduction in total coliforms contained in cow dung and chicken droppings, respectively. Further microbial analysis of the biofertilizer produced from the digestion process showed that pathogenic aerobic and anaerobic micro-organisms, such as species of *Bacillus*, *Clostridium*, *Klebsiella*, *Pseudomonas*, *E. coli*, *Aspergillus* and *Shigella*, were also removed by this process.

Impact on pathogens

The impact of the different treatment processes on pathogenic organisms is clearly very complex, with different organisms being reduced by different amounts by each process, and different concentrations of pathogens occurring in organic wastes from different sources. Total coliforms and *E. coli* are widely used as indicators of faecal contamination, and so provide a simple, standardized method to assess efficacy of different treatment processes (Motlagh & Yang, 2019; Nwaneri et al., 2018). They occur commonly in the intestinal tract of healthy as well as unhealthy animals, and their presence in high concentrations indicates potential health risks due to food and water contaminationv with zoonotic bacterial pathogens (Manyi-Loh et al., 2016).

Impact on crop production

While reduction in pathogens is essential, it is also important that the beneficial impacts of the organic wastes are retained. In Ghana, Torgbo et al. (2018) observed a significant increase in the yield of vegetables cultivated on agricultural soils amended with the recommended rates of N as compost. The head weight of cabbages grown with compost was significantly higher (53.3 g) than those grown with inorganic fertilizer (32.9 g) and the control with no treatment (31.4 g). Similar trends were also observed with the plant height and number of leaves of lettuces. In India, Girija et al. (2019) observed that the application of co-composted faecal sludge from municipal and human wastes resulted in significantly higher yields of crops than untreated farmyard manure, treated faecal sludge, chemical fertilizers and control plots with no treatment. Other parameters such as plant heights, number of leaves, number of beans and weight of beans marginally increased in agricultural soils amended with the co-compost compared with other treatments.

Aims

If the amount of organic waste is limited, as is often the case in rural holdings in developing countries, it is important to consider the impacts on crop yield of organic fertilizers derived from the same amount of starting fresh waste. Therefore, the aim of this paper is to investigate effectiveness of different treatment methods available to households in Nigeria in reducing the burden and transmission of total coliforms and *E. coli* to the soil and harvested food crops, while also considering the impacts on N content of the treated wastes. We hypothesize that *E. coli* and total coliform counts in organic wastes are significantly higher in untreated than in treated organic wastes, and that this difference is transmitted to the soil and lettuces when wastes are applied at the recommended N rate. We also hypothesize that there will be significant differences between treatment methods (anaerobic digestion, composting and burning) with respect to the transmission of *E. coli* and total coliforms to the soil and crops when organic amendments are applied at the recommended N rate. The novelty of this research is in the combined analysis of the impact of the different treatments methods available to farmers in rural Nigeria on the pathogen and nitrogen contents of the manure. This allows direct comparison of the impact of the treatment method on soil and plant contamination if the treated organic waste is then used as a nitrogen source.

MATERIALS AND METHODS

Field trials

Study area

The field work was carried out within Keffi Metropolis of Nasarawa State, Nigeria. Keffi, located in the Middle-belt of Nigeria in the Guinea Savannah, 53 km from Abuja (latitude 8°5°N; longitude 7°52°E; altitude 85 m above sea level (Obumneme et al., 2019).

Field specifications

The experimental field was laid out in a complete randomized block design in a rectangle of 10 × 12 plots, giving a total of 120 plots including 4 replicates ×6 rates of N × 5 sources of N (bioslurry, compost, ash, untreated organic waste and fertilizer). Each plot measured 1 m × 1 m, with a separation distance of 0.5 m between plots. Run-off and cross contamination of plots was avoided by selecting an experimental site with a low gradient and by constructing raised edges on the 0.5-m area between plots to avoid possible run-off during irrigation and rainfall. Standing on plots after sowing was avoided in order to minimize contamination of plots through transfer on footwear.

Source and treatment of cattle manure

Fresh cattle manure was collected from Maliya cattle barn in Keffi, Nigeria in sterile bags and transported to the field site for treatment. The cattle manure was treated by three different common household methods; anaerobic digestion, composting and incineration (burning).

Anaerobic digestion

A floating-drum biogas digester, established and constructed as described by Afriflame (2017) (Figure 1), was used in the anaerobic digestion of the cattle manure. Briefly, the digester was constructed from two polyethylene water storage tanks, one acting as the digestion vessel and the other as the gas collection tank. The final volume of the digester was 2.2 m^3. It was positioned on a solid (brick and cement) base. The feed inlet was situated at the bottom and the digestate outlets was situated at the top. Pipework comprised a 75-mm PVC pipe sealed with epoxy resin. The gas outlet pipe was constructed using a 1.27-cm gas global valve and a brass nozzle. The digester tank was filled with the required volume of feedstock at the ratio of 1:1 manure to water by volume, covered with the gas holder tank and allowed to stand for 30 days. From this, bioslurry was collected for field trials and subsamples were collected to determine coliform and *E. coli* counts.

Composting

A local pit composter, following a design used by rural farmers in Nigeria, was used to compost cattle manure for field trials. A 9 × 9 m square pit was dug to a depth of 1.8 m. Planks were laid beneath the pit to avoid mixing of compost and soil, covered by a large black polythene sheet. The pit was filled with cattle manure (~146 m^3) and was covered with a black nylon sheet to contain heat. Water was added to the compost three times per week (120 dm^3 on each occasion). Once each week, the heap was turned by hand to ensure the compost was mixed and to maintain aeration. The duration of composting was 30 days.

Burning

The cattle manure was treated by burning using a local burn barrel with open lid. Dried cattle manure was heaped inside a metal container with diameter 0.6 m and height 1 m. The dried cattle manure was subjected to slow thermal heating at the surrounding atmospheric temperature of 38°C until completely burnt to produce ash. The ash produced from this

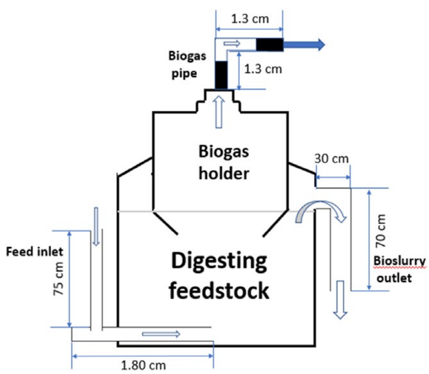

FIGURE 1 Floating drum anaerobic digester; adapted from AfriFlame (2017)

thermal heating was allowed to cool, collected and stored in a sterile polythene bag for use as an organic amendment in the field trial within 24 h. The combustible materials were reduced and settled as ash in the base of the barrel. Reduction in volume was approximately 95%.

Determination of physical and chemical properties of organic manure

Organic matter and organic carbon contents

The percent organic matter, P_{OM} (%), and organic carbon (C), P_{OC} (%), in the waste was determined by a modification of the Springer and Klee method (Ciavatta et al., 1989). Dried samples were ground and oxidized by the addition of 20 ml of 2N $K_2Cr_2O_7$ and 26 ml of concentrated H_2SO_4 at 160 ± 2°C, and manually titrated to determine excess dichromate. The value of P_{OC} was calculated as

$$P_{OC} = \frac{(A - B) \times F \times K}{W} \quad (1)$$

where A is the volume of $FeSO_4$ solution (ml) used in titrating excess $K_2Cr_2O_7$ of the sample, B is the volume of $FeSO_4$ solution (ml) used in titrating excess $K_2Cr_2O_7$ of the blank analysis, F is the correction factor from daily titration of $K_2Cr_2O_7$ solution with $FeSO_4$ (1.30), K is a constant given by the dilution and equivalent C content (0.003 × 100), and W is the weight of the sample (g). The value of P_{OM} was then calculated from P_{OC} as

$$P_{OM} = 0.35 + 1.80 \times P_{OC} \quad (2)$$

Total nitrogen

The total percentage N of the manure samples was estimated by digestion to convert the N compounds to ammonium, followed by distillation and determination of the amount of N by the Kjeldahl method as described by Abrams et al. (2014).

Dry matter

Following Peters et al. (2003), 20 g of organic waste was dried at 110°C to constant mass (24 h). The percentage dry matter content was measured from the weight of the sample before and after drying:

$$P_{dm} = \frac{\left(\left(W_{dw} + W_c\right) - W_c\right) \times 100}{\left(\left(W_{fw} + W_c\right) - W_c\right)} \quad (3)$$

where W_{dw}, W_c and W_{fw} are the weights of dried sample, container and undried sample respectively (all in g).

Application of organic fertilizer

The organic fertilizers were added as an amendment to the plough layer, mixing to a depth of 15–25 cm at six rates, based on the available N contents of bioslurry, compost, ash, untreated organic manure and fertilizer. Precise analyses were not available before the start of trial, but results were later normalized to the recommended rate of fertilizer application (see data analysis). The bioslurry was collected from the outlet of the biogas digester, mixed manually and then applied to each of the plots 1 week before sowing of the lettuces. The compost and ash were applied to each of the plots in the same manner. Fresh untreated cattle manure and inorganic fertilizer were processed and applied to the plots as described earlier.

Cultivation of lettuce

Lettuce seeds were sown 1.27-cm deep in each plot. The experimental plots were irrigated once every 2–3 days until the maturity stage of the crop using tap water. Irrigation with tap water was done using a sprinkler. This method was used to ensure adequate distribution of the irrigation water in the plots. Weeds were removed manually using a hoe and thinning was done at three to five leaf stage in each plot.

Measurement of total coliforms and *E. coli*

Untreated manure

Total coliforms and *E. coli* in untreated manure were determined by placing 5 g fresh cattle manure into 15 ml of sterile diluent, shaking vigorously for 30 s using vortex to produce a homogenous mixture, preparing serial dilutions in universal tubes from three replicate subsamples and plating onto Chromoselect Coliform agar (Sigma Aldrich). Plates were incubated at 37°C for 18–24 h before reading.

Treated manures

Three replicate subsamples of approximately 5 g fresh organic material were collected from each treated material (ash, digestate, compost), diluted in phosphate buffer saline and *E. coli* and total coliforms were determined using the IDEXX Colilert Quanti-Tray 2000 system (IDEXX Laboratories Inc.) according to manufacturer's instructions. Enumeration of total coliforms and *E. coli* in environmental samples by Colilert-18 and plating methods are considered equivalent. No significant difference was observed in concentrations calculated by the two methods by Carner et al. (2013) and Tiwari et al. (2016).

Noncontaminated and contaminated soil

The enumeration of pathogens in unamended and amended soil was carried out using the Colilert method above. A soil auger was used to collect soil samples to a depth of 25 cm from randomly selected locations in each plot, and 5 g soil was collected and diluted in 15 ml of phosphate-buffered saline. The dry matter content of the soil was determined by oven drying the soil samples overnight at 105℃ as described by Pepper et al. (2011). This was used to enumerate the *E. coli* counts per g dry weight of equivalent soil sample which was calculated as described in the previous section.

Harvested lettuces

Fresh harvested lettuce samples were transported to the laboratory unwashed in sterile plastic bags and analysed within 1 h for *E. coli* and coliform counts using the method described by Castro-Rosas et al. (2012): Lettuce leaves were removed from the plant while it was still intact in the plot, using a fresh pair of clean gloves for each plant and taking care not to contaminate them with soil or surface material from the stem. Leafy materials were weighed to give fresh weight of lettuce produced (yield). In order to determine the readily detached *E. coli* and coliform counts of the samples (i.e. those that can be removed during washing and therefore are less likely to be ingested if proper washing procedures are followed), leaf material was divided randomly in half and the "readily detached" and total *E.* coli and coliforms analysed separately. One half was placed into 500 ml sterile 0.01% peptone buffer and shaken for 30 min @ 150 rpm, 10℃. The liquid was then removed and filtered through a 0.25-µm filter and placed onto selective agar (Hi Chrome and MacConkey). This facilitated enumeration of the readily detached *E. coli*/coliforms primarily from the surface of the leaves. The plant material was weighed and dried overnight at 60℃ to determine the dry biomass produced and to allow FIO counts to be expressed on a dry weight basis. The other half of the lettuce leaf material was ground in a mortar with sterile peptone buffer (0.01%) and plated (100 µl) onto selective agar (Hi Chrome and MacConkey) to determine the nondetachable *E. coli* and coliform counts. A subsample was weighed and dried to allow back-calculation of biomass on a dry weight basis.

Data analysis

The mean values were compared using a paired sample *t* test to determine differences *E. coli*/coliform transmission to soil and lettuce crops for different treatments. The data from the field measurements were analysed and transformed to calculate the FIOs reduction, FIOs to N ratio as well as amount of FIOs applied at the recommended N application rate.

Total coliforms and *E. coli* reduction

Changes in total coliforms/*E. coli* were calculated as:

$$\Delta E = \left(E_{\text{start}} - E_{\text{treat}}\right) / W_{\text{treat}} \quad (4)$$

where ΔE is the reduction in total coliforms or *E. coli* due to treatment (MPN per gram), E_{start} is the total coliform or *E. coli* content at the start (MPN), E_{treat} is the total coliform or *E. coli* content after treatment (MPN), and W_{treat} is the dry weight of the treated material (g).

The total coliform and *E. coli* contents of the material at the start of the treatment, E_{start} (MPN), were calculated as.

$$E_{\text{start}} = W_{\text{start}} \times E_{\text{untreat}} \quad (5)$$

where W_{start} is the amount of untreated material used to produce 1 g of treated waste (g) and E_{untreat} is the mean value of total coliform or *E. coli* in untreated manure (MPN g^{-1}).

The amount of starting material W_{start} (g) was calculated as.

$$W_{\text{start}} = 100 / \left(100 - P_{\text{loss}}\right) \quad (6)$$

where P_{loss} is the approximate loss of dry weight material during treatment process (%).

Total coliform and *E. coli* to nitrogen ratio

The mean total coliform or *E. coli* to N ratio of the different treatments, $r_{\text{E:N}}$ (MPN per g of N), was calculated as.

$$r_{\text{E:N}} = \left(E_{\text{treat}} \times 100\right) / N_{\text{treat}} \quad (7)$$

where N_{treat} is the mean N content after treatment (g per 100 g fresh weight of waste)

Total coliform and *E. coli* from waste at recommended nitrogen rate

The volume of treated waste applied in the trial was estimated to give approximately the same rates of N application for the different treatments. Following analysis of the N content of each of the treated wastes, the number of total coliform or *E. coli* in soil following treatment with the recommended N rate as organic waste, $E_{\text{app,rec}}$ (MPN per gram), was back-calculated as.

$$E_{app,rec} = E_{soil} + \left(N_{rec} \times W_1 \times E_{app}\right) / W_{app} \quad (8)$$

where E_{soil} is the amount of total coliform or *E. coli* in untreated soil before amendment (MPN g^{-1}), N_{rec} is the recommended N rate (120 kg ha^{-1}), W_1 is the fresh weight of waste required to supply 1 kg ha^{-1} N (kg ha^{-1}), E_{app} is the amount of total coliform or *E. coli* originating from the applied waste (MPN per gram) observed in the field experiment following application of an amount of waste, W_{app} (kg ha^{-1}).

The amount of waste required to supply 1 kg ha^{-1} N (W_1) was calculated from the mean N content of the waste after treatment, N_{treat} (%),

$$W_1 = 100/N_{treat} \quad (9)$$

The amount of total coliform or *E. coli* originating from the applied waste at a particular time in the growing season was separated from the background organisms in the soil as follows:

$$E_{app} = max\left(\left(E_{obs} - E_{soil}\right), 0\right) \quad (10)$$

where E_{obs} is the number of total coliform or *E. coli* in the waste amended soil, and E_{soil} is the amount of total coliform or *E. coli* in the soil without amendment (both in MPN g^{-1}).

RESULTS

Impact of different household treatment of organic wastes

There was a significant difference in total coliforms and *E. coli* in the cattle manure before and after treatment, and between treatments (Figure 2; $p < 0.01$). Ash contained the lowest concentration of total coliforms and *E. coli*, followed by bioslurry, compost and untreated manure. Total coliforms in ash was 3.0 (±0.6) MPN per gram compared to 88 (±13) MPN per gram in bioslurry, 181 (±19) MPN per gram in compost and 423 (±8) MPN per gram in untreated manure. Similarly, the *E. coli* content of ash was 2.7 (±0.7) MPN per gram compared to 61 (±4) MPN per gram in bioslurry, 144 (±3) MPN per gram in compost and 391 (±3) MPN per gram in untreated manure.

Physical and chemical properties of differently treated organic wastes

The N, organic C, organic matter and dry matter contents of the differently treated organic wastes are shown in Table 1. Percent dry matter in the organic wastes were significantly different in all treated wastes ($p < 0.01$), with the highest content in ash (98.5%) followed by untreated manure, compost and then bioslurry. Percent total N was also significantly different across all the treated wastes ($p < 0.01$), with the highest content in bioslurry while ash contained the least. In order to supply the recommended rate of N to the lettuce crop (120 kg ha^{-1} N), the dry weight application rate would be 20 t ha^{-1} for ash, 7 t ha^{-1} for compost, 4 t ha^{-1} for untreated waste and 2 t ha^{-1} for bioslurry. Percent organic C in bioslurry, compost and untreated manure were not significantly different ($p > 0.05$) but was significantly lower in ash ($p < 0.01$). Similarly, percent organic matter in bioslurry, compost and untreated manure were not significantly different ($p > 0.05$), but was significantly lower in ash ($p < 0.01$).

Reduction of total coliforms and *E. coli* during treatment process

The total coliform and *E. coli* contents of the untreated organic waste were 423 (±8) MPN per gram and 391 (±3) MPN per gram respectively. After treatment, the total coliform contents fell to 3.0 (±0.6) MPN per gram for ash, 88 (±13) MPN per gram for bioslurry and 181 (±19) MPN per gram for compost. Similarly, the *E. coli* content fell to 2.7 (±0.7) MPN per gram for ash, 61 (±4) MPN per gram for bioslurry and 144 (±3) MPN per gram for compost. The treatment process used different amounts of starting material

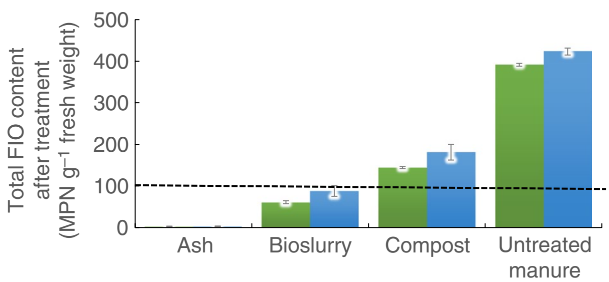

FIGURE 2 Concentration of total coliform and *E. coli* faecal indicator organisms (FIOs) in cattle manure samples before and after treatment. Error bars show standard errors from three replicates. The dotted line indicates United States Environmental Protection Agency (USEPA) *E. coli* limit of < 100 MPN g^{-1} in animal manure destined for land application. Note: USEPA (2003) recommendations for total coliforms in organic manure destined for application as organic fertilizer is < 1000 MPN g^{-1}. Not shown on graph as all treatments are below this level. The term “Ash” refers to cattle manure that has been burnt in a burn barrel, “Bioslurry” to cattle manure that has undergone anaerobic digestion for 30 days, “Compost” to cattle manure that has been composted for 30 days at ambient temperatures in Nigeria with weekly turning to maintain aerobic conditions and “Untreated manure” to cattle manure that has undergone no treatment. Green bars refer to *E.coli*. Blue bars refer to coliforms.
[Correction added on 16 July 2021, after first online publication: the legend for Figure 2 has been updated in this version]

TABLE 1 Physicochemical properties of differently treated organic wastes. Note: SE = standard error of means of six replicates. The term 'Ash' refers to cattle manure that has been burnt in a burn barrel, 'Bioslurry' to cattle manure that has undergone anaerobic digestion for 30 days, 'Compost' to cattle manure that has been composted for 30 days at ambient temperatures in Nigeria with weekly turning to maintain aerobic conditions and 'Untreated manure' to cattle manure that has undergone no treatment

	Total N (%)		Organic carbon (%)		Organic matter (%)		Dry matter (%)	
Organic waste	Mean	SE	Mean	SE	Mean	SE	Mean	SE
Ash	0.59	0.01	1.83	0.004	3.14	0.009	98.5	0.2
Bioslurry	6.41	0.04	1.89	0.004	3.25	0.006	22.7	0.2
Compost	1.7	0.1	1.89	0.004	3.23	0.006	28.4	0.2
Untreated manure	2.8	0.1	1.88	0.004	3.23	0.003	48.0	0.6

to produce the same quantity of treated waste, depending on the percentage reduction of the waste during treatment. Assuming the observed reduction in dry matter by ~95% during burning, ~50% during anaerobic digestion and ~40% during composting, this represents a reduction in pathogens of 99.97% during burning, 92% during anaerobic digestion and 78% during composting. The concentration of total coliforms and *E. coli* in the treated waste were significantly reduced in all treatments, and most of all by burning (Figure 3).

Transfer of total coliforms and *E. coli* to soil following use as a nitrogen fertilizer

Figure 4 shows the ratio of total coliforms and *E. coli* to N for the differently treated organic wastes. There was a significant

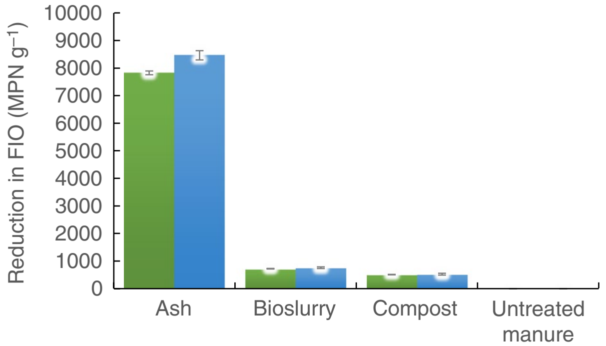

FIGURE 3 Reduction in total coliforms and *E. coli* due to treatment. Note, this shows the reduction in organisms originating from the total amount of starting material needed to produce 1g of treated waste. Error bars indicate standard errors from measurement of three sample replicates. FIO = faecal indicator organisms. The term "Ash" refers to cattle manure that has been burnt in a burn barrel, "Bioslurry" to cattle manure that has undergone anaerobic digestion for 30 days, "Compost" to cattle manure that has been composted for 30 days at ambient temperatures in Nigeria with weekly turning to maintain aerobic conditions and "Untreated manure" to cattle manure that has undergone no treatment. Green bars refer to *E.coli*. Blue bars refer to coliforms. [Correction added on 16 July 2021, after first online publication: the legend for Figure 3 has been updated in this version]

difference in the *E. coli*: N ratio of all treated wastes compared to untreated waste. The total coliforms: N ratio of all treated wastes except for compost were also significantly different to untreated waste. The ratio of total coliforms and *E. coli* to N in bioslurry was significantly lower than in ash, compost or untreated waste.

Impact of applying nitrogen as differently treated wastes on pathogens in soil and crops

Back-calculating the coliform and *E. coli* content of the soil to correspond to application of the recommended rate of 120 kg ha^{-1} of N resulted in a significant increase in the *E. coli* content of the soil immediately after amendment for all treatments except bioslurry, as indicated by paired sample *t* test ($p > 0.05$) (Figure 5a). However, there was no significant increase in the total coliform content of the soil immediately after amendment for any of the treatments except ash ($p < 0.01$) (Figure 5b). Although the total coliform and *E. coli* content of ash was low, the very low N content of the ash meant that a large quantity of ash had to be applied, resulting in the high levels of total coliforms and *E. coli* being added to the soil (Figure 4). The back-calculated total coliform content of soil immediately after application of ash was significantly higher than if the same amount of N had been applied as bioslurry ($p < 0.05$), compost ($p < 0.05$) or untreated manure ($p < 0.05$). Similar significant increase was also observed in the *E. coli* of the soil immediately after application of ash if the same amount of N had been applied as bioslurry ($p < 0.01$) or untreated manure ($p < 0.05$) but there was no significant difference compared to application as compost ($p > 0.05$). The total coliform and *E. coli* contents were not significantly different for application of compost or untreated manure ($p > 0.05$), but if N was applied as bioslurry, the total coliform and *E. coli* contents of the soil were significantly lower than in all other treatments ($p < 0.05$).

Following organic amendment, soils showed an immediate decline in total coliform and *E. coli* counts irrespective of organic waste treatment. The reduction in *E. coli* due to

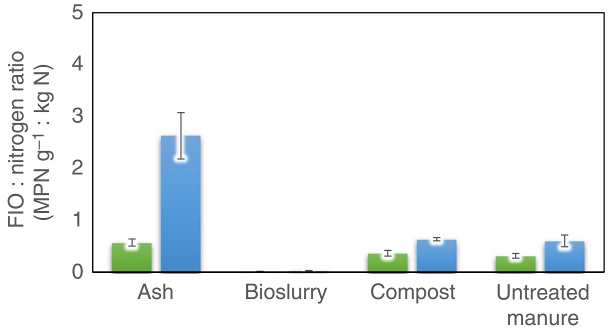

FIGURE 4 Total organism to nitrogen ratio of treated wastes for coliforms and *E. coli*. Error bars show standard errors of three replicates. Note FIO = faecal indicator organisms. The term "Ash" refers to cattle manure that has been burnt in a burn barrel, "Bioslurry" to cattle manure that has undergone anaerobic digestion for 30 days, "Compost" to cattle manure that has been composted for 30 days at ambient temperatures in Nigeria with weekly turning to maintain aerobic conditions and "Untreated manure" to cattle manure that has undergone no treatment. Green bars refer to *E.coli*. Blue bars refer to coliforms. [Correction added on 16 July 2021, after first online publication: the legend for Figure 4 has been updated in this version]

treatment of organic waste was significant ($p < 0.05$) at all sampling times except immediately after harvest, when the values were low in both the untreated and treated amendments. For bioslurry, the *E. coli* content of the soil was not significantly different to background levels immediately after amendment ($p < 0.01$), whereas *E. coli* did not fall to this level for the other treatments until harvest. For the untreated manure, the *E. coli* content of the soil remained significantly higher than background levels even after harvest ($p < 0.01$). By contrast, the reduction in total coliforms due to treatment of the organic waste was not significant ($p > 0.05$) at any sampling time. The total coliform content of the soil was not significantly different to background levels immediately after amendment for any treatment except ash ($p < 0.01$), and by harvest time, there was no significant difference in background levels in any treatment ($p > 0.05$). This was reflected in the values of total coliforms and *E. coli* back-calculated for harvested lettuces grown on soil with the recommended N rate of differently treated wastes.

DISCUSSION

Contamination of lettuces grown in soils fertilized with untreated organic wastes

Our results showed that when untreated cattle manure was applied to soils, the levels of *E. coli* in the lettuce crop were significantly higher than the background level. Many other authors have also observed that cattle manure serves as a reservoir for intestinal pathogens, such as verocytoxigenic *E. coli*. Harris et al. (2003) reported the contamination of food crops with pathogenic micro-organisms as a result of continued use of untreated animal manure as fertilizers in Nigeria. Uzeh and Adepoju (2013) and Moses et al. (2016) identified outbreaks of gastroenteritis with consumption of *E. coli*-contaminated vegetables grown on soil fertilized with animal wastes. Therefore, application of untreated animal manure to agricultural soil increases the risk of contamination of soil and food crops, and effective treatment before application to the soil is therefore of great importance.

By contrast, our results showed no increase over the background levels in total coliforms in lettuces as a result of application of untreated manure. This differed from the findings of Szczech et al. (2018), who observed that the use of animal manures as organic fertilizer resulted in significantly increased levels of total coliforms in lettuces. Similarly, in Southern Benin, other vegetable crops (leafy eggplant, tomato and carrot) were found to be heavily contaminated with coliforms after application of untreated animal manure as organic fertilizer on field trials (Atidelga et al., 2016). Shenge et al. (2015) observed a high median concentration of coliforms in excess of 1000 MPN per gram on all field- and market-sourced vegetable crop in North-western Nigeria, with preharvest microbial contamination in the field from the use of untreated animal manure being suggested as the predominant cause of the high coliform counts (Shenge et al., 2015). The nonsignificant difference in this study may be due to the low levels of total coliforms in the untreated manure; this was well below EPA NSW (1997), USEPA (2003) and Canadian Council of Ministers of the Environment (CME) (2005) recommended guidelines for total coliforms in organic manure destined for application as organic fertilizer (<1000 MPN per gram). It may also be due to high background levels of total coliforms in soils due to previous use of untreated manures at the site.

Treatment of cattle manure for pathogen reduction

The different treatment methods available to households in Nigeria all showed significant reduction in the total coliforms and *E. coli* content of the cattle manure. Treatment by burning led to greatest reductions of all treatments tested. Burning is commonly practiced in many rural areas of Nigeria as it is the easiest way to dispose of contaminated material. However, if the aim is to use the treated organic waste as an organic fertilizer, burning may not be the best option because the loss of nutrients means application rates would have to be increased in order to meet the recommended rate, increasing addition of FIOs to the soil.

Use of anaerobic digestion to treat cattle manure resulted in a significantly greater reduction in the concentration of

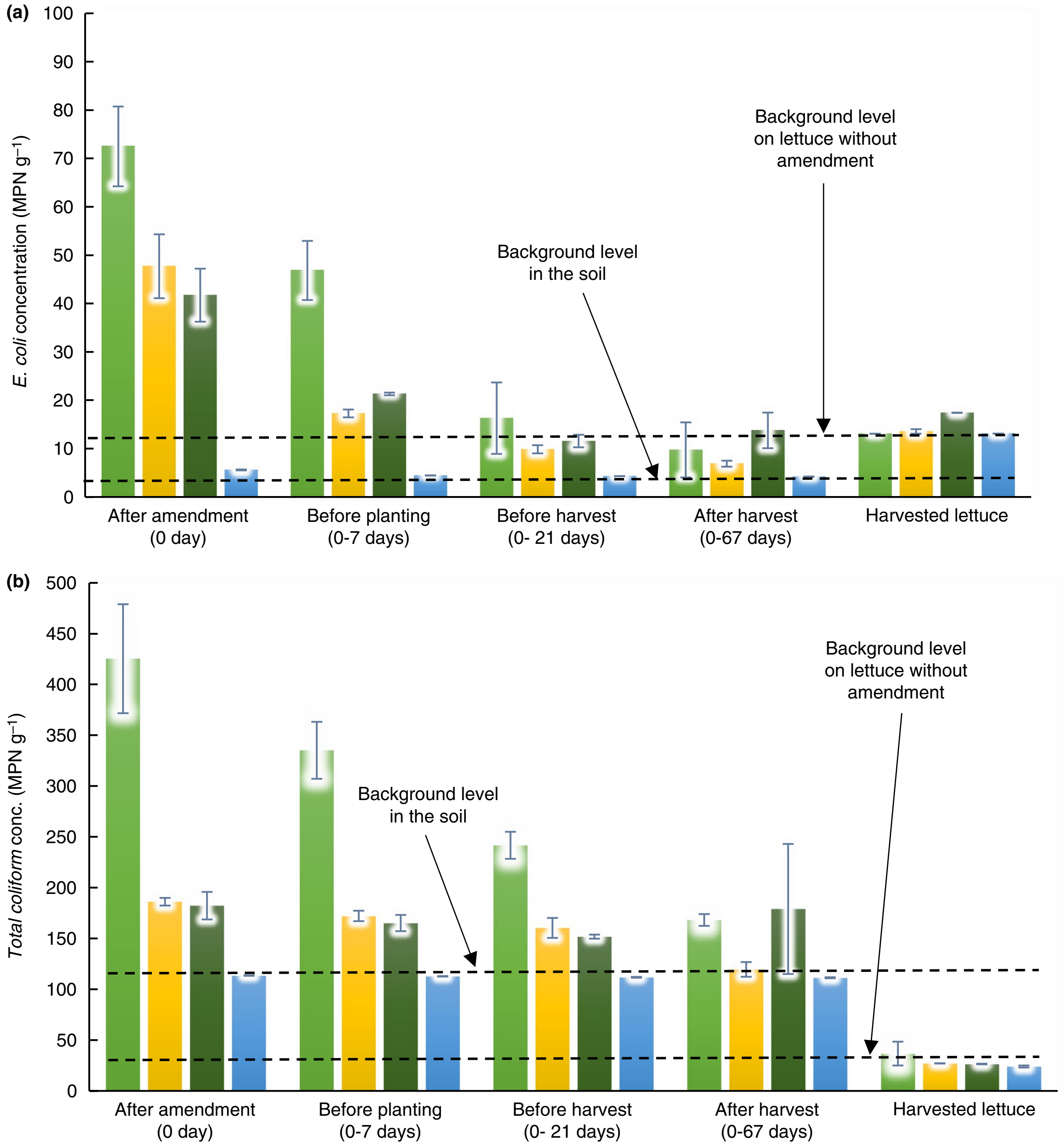

FIGURE 5 Impact of applying the same amount of nitrogen on the transfer of (a) E. coli and (b) total coliforms at different sampling points with the application of 120 kg ha^{-1} of differently treated and untreated manure. Error bars represent standard errors of the mean of three replicate samples. The dotted line represents the background total coliforms and E. coli content in soils before the application of the different treated and untreated animal wastes. The term "Ash" refers to cattle manure that has been burnt in a burn barrel, "Bioslurry" to cattle manure that has undergone anaerobic digestion for 30 days, "Compost" to cattle manure that has been composted for 30 days at ambient temperatures in Nigeria with weekly turning to maintain aerobic conditions and "Untreated manure" to cattle manure that has undergone no treatment. Green bars refer to ash, yellow bars to compost, dark green to untreated manure and blue bars refer to bioslurry.
[Correction added on 16 July 2021, after first online publication: the legend for Figure 5 has been updated in this version]

total coliforms and *E. coli* than composting (Figure 2). The effectiveness of anaerobic digestion in reduction of pathogenic organisms is dependent on temperature, constituents of the feedstock and retention time (Chen et al., 2012; O'Reilly et al., 2009). The design of the digester and maintenance of anaerobic conditions are also important factors (Avery et al., 2014). This study used a floating drum digester to treat wastes at temperatures of 36–37°C for 30 days. These conditions were effective in reducing the FIOs present in the cattle manure. It may be possible to adopt a shorter retention time and still demonstrate effective removal of FIOs if the manure has a higher C:N ratio (Font-Palma, 2019) or if the digester is operated at a higher temperature (Smith et al., 2005). In sub-Saharan Africa, hydraulic retention time for digesters is typically ~40 days (Smith et al., 2013).

Note that in order to determine effectiveness of anaerobic digestion in reducing total coliforms and *E. coli* compared to other treatments, digestion was managed as a batch operation with no daily addition of new feedstock. This was done to allow the total coliform and *E. coli* content of the feedstock to be accurately characterized; if feedstock had been added daily, the microbial contents would have changed on a daily basis, making it difficult to determine the starting levels of total coliforms and *E. coli* in the untreated manure. Assuming a continuous digester has minimal mixing, then bioslurry leaving the digester will have been undergoing anaerobic digestion for the period of the hydraulic retention time in the digester. Composting also significantly reduced the total coliform and *E. coli* content compared to untreated wastes, although by less than anaerobic digestion. Composting is a cheap, accessible and effective method for on-site treatment of wastes, and so is commonly used in most parts of Africa (Edwards & Araya, 2011). Its efficacy in reducing FIOs and other zoonoses in animal manures has been widely demonstrated (e.g. Asses et al., 2019; Avery et al., 2012; Larney et al., 2003; McCarthy et al., 2011; Wichuk & McCartney, 2007).

There are currently no regulations for manure treatment, anaerobic digestion or organic manure application to agricultural land in Nigeria (Ndambi et al., 2019) and therefore international standards were used for comparison. While all three treatment methods significantly reduced the *E. coli* content of the manure, the mean concentration in compost (144 [±3] MPN per gram) remained above United States Environmental Protection Agency (USEPA, 1993), New South Wales Environmental Protection Agency (NSW EPA, 1997) and New Zealand Waste water Association (NZ WWA, 2003) guidelines for organic manure destined for agricultural land of <100 MPN per gram. By contrast, the concentrations of *E. coli* in ash (2.7 [±0.7] MPN per gram) and bioslurry (61 [±4] MPN per gram) were well below this limit. The total coliform content of the manure was below the acceptable safety limits in all three treatments; the mean concentrations in ash (3.0 [±0.6] MPN per gram), bioslurry (87.9 [±12.8] MPN per gram and compost (181.3 [±19.02] MPN per gram) were below the USEPA (2003), Canadian Council of Ministers of the Environment (CME) (2005) and NSW, EPA (1997) recommended guidelines of <1000 MPN per gram for organic manure destined for application as organic fertilizer.

Transmission of *E. coli* and coliforms to soil as a result of organic wastes application

Many authors (for example Ingham et al.,2004, Islam et al.,2004 and Sheng et al., 2019), reported the persistence of faecal coliforms and *E. coli* in soil amended with organic wastes for long periods of time. This increases the risk of potential exposure and contamination of the crop, so it is important to understand how treatment of organic wastes before application affects the concentration of faecal coliforms and *E. coli* present in the soil. Our results show that the application of the differently treated wastes in the quantities required to provide the recommended rate of N resulted in a significant increase in contamination of the soil with total coliforms and *E. coli* over the background levels in all cases except for bioslurry. By contrast, other studies have observed significant increases in *E. coli* for all the forms of organic waste considered here; Goberna et al. (2011) observed significant counts of *E. coli* in a pot experiment amended with untreated cattle manure compared to soil amended with anaerobically digested fresh manure, Jensen et al. (2013) reported increased levels of *E. coli* in the field as a result of application of cattle bioslurry, while Zhen et al. (2014) demonstrated that the application of manure-based compost led to a significant increase in the numbers of *E. coli* in the soil in pot experiments. Similarly, an increase in the total coliform count in agricultural soils has been observed following the application of both compost (Sheng et al., 2019) and bioslurry (Saunders et al., 2012).

The lower levels of coliform and *E. coli* contamination observed with bioslurry in our trials may have been due to the type of organic amendments, rate of application and the specific experimental conditions. Mtapuri-Zinyowera et al. (2009) observed 81%–95% decay in the soil in pathogens originating from manures due to exposure to sunlight and Sharma et al. (2019) observed a relationship between temperature and survival of micro-organisms in the soil, Therefore, it is important to investigate pathogen die-off for the specific weather conditions of the country of interest. Soil characteristics can also influence FIO and pathogen survival. For example, Brennan et al. demonstrated the impact of clay type and content on survival of a range of enteropathogens including *E. coli* O157:H7 (Brennan et al., 2014). Longer survival times for pathogens have also been observed in soils amended with higher levels of organic manures (Çekiç et al., 2017). Studies by Jiang et al. (2002) suggested that the nutrient content of organic manures influences the decay of pathogens in the soil due to the antagonistic effects of micro-organisms in the manure amended soil. They observed rapid die-off of pathogens in soils amended with high nutrient and pathogen contents at normal growing temperatures. In our study, we controlled for this by linking the rate of organic amendment to the N content of the treated waste and the recommended N application rate; if higher volumes of organic wastes had been applied, different results would be expected.

Contamination of lettuces grown in soils with treated organic wastes

While we observed a significant increase over background levels in *E. coli* concentrations in lettuces grown in soils amended with untreated manures, there was no significant increase for soils amended with any of the treated manures at the recommended N rate, whether this was ash, bioslurry or compost. Interestingly, despite the significantly higher counts of total coliforms and *E. coli* observed in amended soil from the ash treatment immediately after harvest, the *E. coli* counts on lettuces grown on soils amended with ash were significantly lower than on soils amended with compost or untreated manure ($p < 0.05$), suggesting the persistence of the population had been weakened by burning. Similarly, although there was no significant difference in the *E. coli* counts between the composted and untreated manures immediately after application, the counts on harvested lettuces grown with compost were significantly lower than for untreated manure ($p < 0.05$), suggesting that the composting process had reduced the persistence of the *E. coli*.

The significantly lower concentration of *E. coli* on lettuce plants grown on soil amended with the treated cattle manure demonstrates the effectiveness of the treatment methods in reducing the risks associated with the use of organic fertilizers. Lettuces are usually consumed uncooked. The level of contamination of this vegetable with *E. coli* is of public concern because the presence of *E. coli* indicates faecal contamination as well as indicating likely presence of zoonotic bacterial pathogens (Jensen et al., 2013). Uzeh and Adepoju (2013) reported outbreaks of gastroenteritis linked to consumption of *E. coli*-contaminated fresh vegetables grown on soil amended with fresh cattle manure in Nigeria. Also, Rosen and Allan (2007) in a study reported that a total of 70% of the total foodborne in the USA and 75% of the total fresh produce outbreaks in Brazil were attributed to fresh vegetables.

According to Espigol et al. (2018), there are no set standards for coliform levels on fresh fruits and vegetables due to the inherently high concentrations of total coliforms (which represent a more diverse group of FIOs than *E. coli*) on these food crops. However, total coliform contents of lettuces grown on soils amended with both untreated and treated manure, as well as those grown without organic amendment, were well above the Food and Drug Administration (1998) acceptable minimum limit of <3 MPN per gram for coliform and *E. coli* on food for human consumption, so may constitute a significant human health risk. This could be due to the long-term build-up of *E. coli* and total coliforms in the soil due to application of untreated manures in previous years. Comparison of counts in lettuces grown with and without organic amendments shows no significant increase in either *E. coli* or total coliforms over the background for any of the treatment methods. This suggests all three treatments are effective at reducing contamination of lettuce crops with *E. coli* and total coliforms.

CONCLUSION AND RECOMMENDATIONS

In conclusion, there was significant reduction in the *E. coli* content of cattle manure following treatment. Treatment by burning and anaerobic digestion met international regulatory standards for discharge into agricultural soil, whereas treatment by composting did not. Therefore, composting should be improved to avoid spread of pathogens to the wider environment.

Burning was the best method for reducing pathogens in manure, but also reduced the concentration of N more than the other treatments. If ash was applied at the recommended N rate, the pathogens added to the soil were significantly higher than for either anaerobic digestion or composting. Application of bioslurry at the recommended N rate resulted in *E. coli* levels that were not significantly higher than the background. Therefore, if the intention is to use organic waste as an organic fertilizer, anaerobic digestion is the most effective form of treatment for combined microbiological sanitization and nitrogen content.

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CONFLICT OF INTEREST

No conflict of interest declared.

AUTHORS CONTRIBUTION

The contribution of the authors in developing this manuscript is outlined as follows:

1. V. A. Chukwu (Corresponding author) contributed to experimental design and set up of field trial. Conducted sample collection and laboratory analysis of collected samples. Carried out data and statistical analysis of field measurements as well as drafting of the main manuscript.
2. J. U. Smith (Co-author) contributed to the experimental design of the field trial, data and statistical analysis of the field measurements. Also contributed to drafting of the manuscript.

3. N. Strachan (Co-author) contributed to experimental design of the field trial and drafting of manuscript.
4. L. Avery (Co-author) contributed to experimental design of the field trial, laboratory works, data analysis and drafting of manuscript.
5. S. Obiekezie (Co-author) assisted in setting up of the field trial, sample collections and laboratory work. Also commented on the final draft of the manuscript.

ORCID

Vince A. Chukwu https://orcid.org/0000-0001-9079-5801

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