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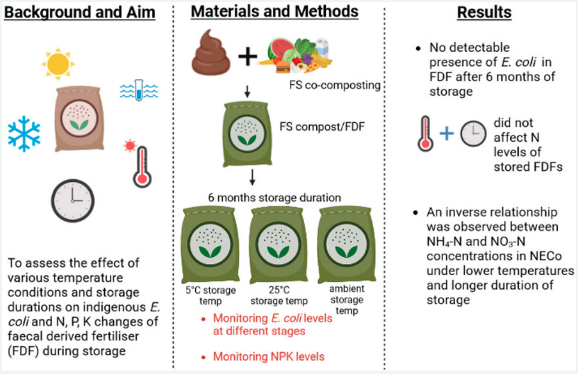
# Storage duration and temperature affect pathogen load, heavy metals, and nutrient levels in faecal derived fertiliser

Eric Gbenatey Nartey[a,b], Ruben Sakrabani [a], Sean Tyrrel [a] and Olufunke Cofie[b]

[a]Cranfield University, Bedford, UK; [b]International Water Management Institute, Accra, Ghana

**ABSTRACT**

There are debates regarding the safety of faecal derived fertiliser (FDF) due to notions that harmful substances may persist at undetectable levels. A major concern is the recolonisation of indigenous pathogens and nutrient changes while undergoing storage. Abiotic factors such as duration and temperature on indigenous pathogen re-growth and nutrient during FDF storage have received little research attention. In this study, we assess the effect of varying storage temperature conditions and duration on indigenous *E. coli* re-growth and NPK changes of different FDF (enriched co-compost, NECo and co-compost, Co) during storage. A 2 × 3 × 6 factorial design was used with factors: fertiliser, temperature, and duration. The factorial had 36 experimental conditions in a completely randomised design with three replications. FDF samples were collected monthly for 6 months and analysed for pH, EC, organic carbon, N, $NH_4$-N, $NO_3$-N, P, K, *E. coli*, and total coliform. Findings show storage temperature and duration did not affect indigenous *E. coli* re-growth and total N in stored NECo and Co. However, $NH_4$-N concentrations of NECo decreased between 27% and 55% with increasing duration of storage at lower temperatures (5°C and 25°C). The significance of this study for the FDF industry is that it is safe after storage and longer storage do not necessarily influence nutrient losses in stored FDF. Future studies are recommended to investigate the effect of moisture on stored FDF.



To assess the effect of various temperature conditions and storage durations on indigenous *E. coli* and N, P, K changes of faecal derived fertiliser (FDF) during storage

- Monitoring *E. coli* levels at different stages
- Monitoring NPK levels

- No detectable presence of *E. coli* in FDF after 6 months of storage

did not affect N levels of stored FDFs

- An inverse relationship was observed between $NH_4$-N and $NO_3$-N concentrations in NECo under lower temperatures and longer duration of storage

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**KEYWORDS**
Faecal derived fertiliser; storage; *E. coli*; nutrients; temperature

## 1. Introduction

Co-compost produced from organic waste streams must satisfy key product safety and quality requirements to enable them to meet the needs of both organic and conventional agricultural farming. For faecal sludge (FS) based co-composts, comprehensive information is required for every stage of the production value chain to communicate safety and quality due to potential transmission of indigenous pathogen to humans [1,2]. FS-based co-compost also referred to as faecal derived fertiliser (FDF) is a promising alternative to mineral fertilisers in sub-Saharan Africa (SSA) as it contributes to sustainable waste management and sustainable agriculture [3–6]. It is more relevant and urgent now because of the convergence of several factors such as geopolitical risks and conflicts around natural resources, climate change urgency, competition in the markets for natural resources, the finite nature of some resources such as phosphate rocks [7], and the growing scientific and technological know-how, makes now the right time [8,9] to encourage co-composting.

The aerobic co-composting method has been proven as a reliable and low-cost technology for the treatment and conversion of organic waste including FS and food

**CONTACT** Ruben Sakrabani r.sakrabani@cranfield.ac.uk Cranfield University, College Rd, Wharley End, Bedford MK43 0AL, UK

waste (FW) into FDF prior to agricultural use [10–12]. This method has been used as a practical and effective way for stabilising organic matter and reducing human pathogen in FS [12–14]. One of the reasons for the continuous debate regarding the safety of FDF is the notion that, even though treatment via co-composting is completed, heavy metals, harmful toxins and pathogens may still be present at undetectable levels. Under favourable conditions, some pathogens may multiply to re-contaminate the product. Thus a major concern of FDF usage is the possibility of human pathogen re-contamination or recolonisation from either external sources or indigenous re-growth to hazardous levels [15–17] as well as losses in nutrient quality characteristics [18].

In the production value chain, when FDF is produced and not used immediately, it is placed in storage until the next use. The duration of storage is dependent on some parameters such as demand & supply, distribution & marketing, and climatic conditions. Storage itself can take place under different conditions in warehouses, on farmers' fields, etc. Since FDF is just like any other co-compost material with crumbly, fine, brown, and light characteristic material, the duration and conditions of storage may expose it to changes in texture, physico-chemical, and pathogen characteristics. The changes in these characteristics during storage depend on abiotic (temperature, pH, humidity) [19] and biotic (composition and diversity of the microbial community) factors. Several studies have been carried out on the co-composting dynamics of FS [20–22]. But very few recent studies have been carried out on the storage of FDF [23]. Some studies carried out on co-compost and compost-based amendments analysed the role of intrinsic abiotic factors such as moisture, temperature, and available nutrients on pathogen re-growth [24]. Other authors have exclusively focused on the role of biotic factors such as indigenous microflora [25,26] in the suppression of mostly seeded or inoculated pathogens.

However, the effect of extrinsic abiotic factors such as storage duration and temperature conditions on potential indigenous pathogen re-growth and changes in FDF nutrient as well as other properties during storage has received very little or no research attention [16,23]. This study hypothesises that extrinsic abiotic factors do alter pathogen, nutrient, and heavy metal characteristics of FDF in storage. In SSA, where FDF is being promoted, as a solution to sustainable waste management and agriculture, there remains a dearth of information on FDF characteristics in storage. No study has investigated the effect of abiotic storage conditions on inherent *E. coli* re-growth and N, P, K dynamics of FDF during storage. Therefore, in this study, the aim was to assess the effect of varying storage temperature conditions and storage duration on indigenous *E. coli* re-growth levels and N, P, K changes of different faecal derived fertilisers during storage.

## 2. Materials and methods

### 2.1. Faecal derived fertiliser production

The FDFs for this study were prepared at the Jekora Ventures Limited (JVL) – Yilo Krobo Municipal Assembly (YKMA) Recycling Plant, in Akorley, Somanya (latitude 60.00′N and 00.30′N and between longitude 00.30′W and 10.00′W [27]) in the YKMA of Ghana. The plant is a local commercial FS and solid waste treatment operation that had been producing FDF and biomass waste briquettes since 2020. A uniqueness of the JVL-YKMA Recycling Plant is that it is a 'one stop shop' from FS dislodging, treatment, to product as co-compost (FDF) all happening in one place and allows ease of monitoring of the various processes. The FDF was prepared from raw FS collected by vacuum trucks (from households and other institutions) and source segregated food waste (FW) sourced from local markets and restaurants. The raw FS and FW were taken through pre-treatment process of dewatering and shredding, respectively before the co-compost piles were formed in replicates and in batches. In a batch, there were four replicates of co-compost piles built with FW and dewatered FS (DFS) at a ratio of 3:1 w/w. Each pile was approximately 2.0 tons in weight and of the size 1.5 × 10 m (height × base circumference) at the start. The process lasted for 100 days and the matured co-compost post processed by spreading out the piles to air dry until moisture content was <15%. A detailed description of the co-composting process is described in Nartey et al. [28].

The FDF that is matured co-composts (Co) was divided into two parts and one part enriched with ammonium sulphate mineral fertiliser to attain 3% total N content (NECo), following methods described by Adamtey et al. [23]. The moisture content of the FDFs at the end of production was maintained between 10% and 15%. Both Co and NECo were filled into labelled 5 kg sized plastic sacks lined with transparent polyethylene material (a reproduction of the real life 50 kg sized sacks used for bagging FDF from the recycling plant). The sacks in each group were randomly further divided into two sub-groups before placing in storage.

### 2.2. Experimental design

A 2 × 3 × 6 factorial design was employed. The factors were fertiliser type (F), storage temperature (T), and storage duration (D). There were two types of FDFs (F1 = NECo and F2 = Co) which were studied parallel to each other, three levels of temperature conditions (T1 = 5°C, T2 = 25°C and T3 = ambient temperature), and six levels of the storage duration (D1 = 1 month, D2 = 2 months, D3 = 3 months, D4 = 4 months, D5 = 5 months, and D6 = 6 months). The factorial design table is shown in Figure 1.

The 2 × 3 × 6 factorial had 36 experimental conditions in a completely randomised design (CRD) with three (3) replications. Thus a total of 108 experimental treatments were conducted with the FDFs. The 5°C and 25°C storage temperatures were achieved by placing the bags in large storage cold rooms at Noguchi Memorial Institute for Medical Research (NMIMR) and International Water Management Institute research labs in Ghana, respectively. The ambient storage conditions were achieved by placing the bags under a built shed over a concrete platform. The ambient temperatures for the duration of the entire experiment ranged from 24°C to 30°C.

### 2.3. Sampling and analyses

The Co and NECo samples were collected on monthly basis (at the end of each month of storage) from each treatment factor and analysed for pH, electrical conductivity (EC), organic carbon, total N, $NH_4$-N, $NO_3$-N, total P, total K, *E. coli*, and total coliform. Heavy metals and trace elements: Ca, Mg, Mn, Cu, Zn, Fe, Pb, Cd, Cr, Hg, Ni, As, and Se were determined before and after the experiment. Total N was determined by the modified Kjeldahl method described in Black [29]. Ammonium ($NH_4$-N), Nitrate ($NO_3$-N), Total P and K were determined by methods, as described in Okalebo et al. [30]. The pH and EC were measured using 1:5 and 1:10 compost: water w/v ratios, respectively, described in USDA and USCC [31]. Organic carbon (OC) was determined by the Walkley and Black [32] method. The *E. coli* and total coliform counts were done using the spread plate method [33]. Helminth egg was determined by the flotation and sedimentation method following a modified USEPA method [34].

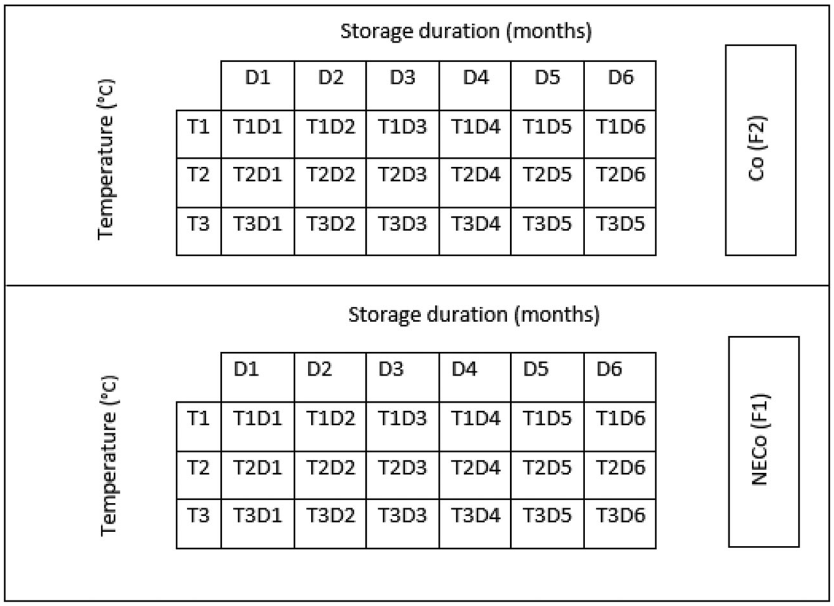
### 2.4. Statistical analysis

Data were subjected to analysis of variance (ANOVA) using Genstat 12th edition statistical package. The statistical analysis was done for each FDF separately or in parallel to the other and not comparing each against other. Treatment means found to be significantly different from each other at ($p < 0.05$) were separated by the Least Significant Differences (LSD) tests.

## 3. Results

### 3.1. Characteristics of faecal derived fertiliser before and after enrichment

The characteristics of the various FDFs (Co and NECo) used in the study are shown in Table 1 and compared with some international organic/compost standards. The concentrations of available (avail.) P, Ca, Cu, EC, Hg, Mg, Ni, Zn, total K and P did not change from what was observed in Co after enrichment to NECo



Storage duration (months)

Temperature (°C)

| | D1 | D2 | D3 | D4 | D5 | D6 |
|---|---|---|---|---|---|---|
| T1 | T1D1 | T1D2 | T1D3 | T1D4 | T1D5 | T1D6 |
| T2 | T2D1 | T2D2 | T2D3 | T2D4 | T2D5 | T2D6 |
| T3 | T3D1 | T3D2 | T3D3 | T3D4 | T3D5 | T3D5 |

Co (F2)

Storage duration (months)

Temperature (°C)

| | D1 | D2 | D3 | D4 | D5 | D6 |
|---|---|---|---|---|---|---|
| T1 | T1D1 | T1D2 | T1D3 | T1D4 | T1D5 | T1D6 |
| T2 | T2D1 | T2D2 | T2D3 | T2D4 | T2D5 | T2D6 |
| T3 | T3D1 | T3D2 | T3D3 | T3D4 | T3D5 | T3D6 |

NECo (F1)

**Figure 1.** Factorial design table.

(Table 1). As expected, total N, $NH_4$-N and $NO_3$-N were significantly higher in the NECo than in Co. The pH and avail. K concentration in the Co reduced after enrichment while heavy metals such as Pb, Cr, Cd, and As concentrations also reduced significantly at $p < 0.05$ after enrichment. *E. coli* levels were below detectable limits in the FDFs (Table 1).

The heavy metals, Pb, Cr, Cd, and As concentrations were observed to have 46%, 55%, 79%, and 74% lower concentrations, respectively, in the NECo after enrichment while Fe, Mn, and Se concentrations were observed to be 25%, 83%, and 100% higher, respectively after enrichment. Some of the heavy metal levels measured in Co and NECo were below the limits set by ECN-QAS and Ecocert standards (Table 1).

## 3.2. Effects of storage temperature and duration on indigenous E. coli and total coliform counts in stored faecal derived fertilisers

### 3.2.1. Main effect of storage temperature on NECo and Co

The main effect of storage temperature was significant at $p < 0.05$ for total coliform in both NECo and Co. The mean total coliform count observed for NECo was in the order of 0.8 > 0.4 > 0.1 log unit for 25°C, 5°C, and ambient storage temperatures, respectively. There were no significant differences between 25°C and 5°C as well as between 5°C and ambient temperatures. However differences were significant between ambient storage temperature and 25°C temperature. Ambient and 5°C storage temperature conditions supported lower coliform counts. The temperature effect on *E. coli* was not observed as *E. coli* was absent (below detection limit).

The effect of storage temperature on mean total coliform count observed for Co was in the order of 2.7 > 2.5 > 2.0 log unit for 5°C, 25°C and ambient storage temperatures, respectively. There was significant difference in total coliform between 5°C and ambient storage temperatures. In the case of Co, storage under ambient conditions supported less total coliform counts than colder temperatures such as 5°C.

**Table 1.** Physico-chemical characteristics of the faecal derived fertiliser types.

| Parameter | Co | NECo | ECN-QAS | ECOCERT Standard |
|---|---|---|---|---|
| pH (1:5) | $8.7^{a}$ | $6.1^{b}$ | – | – |
| EC (1:10) (mS/cm) | $5.3^{a}$ | $5.6^{a}$ | – | – |
| Total N (%) | $1.04^{b}$ | $3.03^{a}$ | – | – |
| $NH_4$-N (mg/kg) | $1300.0^{b}$ | $509200.0^{a}$ | – | – |
| $NO_3$-N (mg/kg) | $1200.0^{b}$ | $231000.0^{a}$ | – | – |
| Org. C (%) | $13.6^{b}$ | $17.9^{a}$ | – | – |
| C:N ratio | $13.3^{a}$ | $5.9^{b}$ | – | – |
| Total P (%) | $1.8^{a}$ | $1.8^{a}$ | – | – |
| Avail. P (mg/kg) | $6.5^{a}$ | $4.0^{a}$ | – | – |
| Total K (%) | $1.8^{a}$ | $2.1^{a}$ | – | – |
| Avail. K (mg/kg) | $0.14^{a}$ | $0.02^{b}$ | – | – |
| Ca (%) | $0.96^{a}$ | $1.22^{a}$ | – | – |
| Mg (%) | $0.33^{a}$ | $0.35^{a}$ | – | – |
| Mn (mg/kg) | $14.9^{b}$ | $27.3^{a}$ | – | – |
| Cu (mg/kg) | $6.2^{a}$ | $7.9^{a}$ | 300.0 | 70.0 |
| Zn (mg/kg) | $531.5^{a}$ | $567.2^{a}$ | 600.0 | 200.0 |
| Fe (mg/kg) | $289.3^{b}$ | $362.7^{a}$ | – | – |
| Pb (mg/kg) | $66.7^{a}$ | $35.7^{b}$ | 40.0 | 25.0 |
| Cd (mg/kg) | $6.1^{a}$ | $1.3^{b}$ | 1.3 | 0.7 |
| Cr (mg/kg) | $66.3^{a}$ | $29.6^{b}$ | 60.0 | 70.0 |
| Hg (mg/kg) | $0.29^{a}$ | $0.13^{a}$ | 0.45 | 0.4 |
| Ni (mg/kg) | $0.01^{a}$ | $0.02^{a}$ | – | – |
| As (mg/kg) | $1.08^{a}$ | $0.28^{b}$ | – | – |
| Se (mg/kg) | $0.02^{b}$ | $0.04^{a}$ | – | – |
| *E. coli* (CFU/g) | $<1.0^{a}$ | $<1.0^{a}$ | – | – |
| Total coliform (CFU/g) | $1.1\times10^{4a}$ | $<1.0^{b}$ | – | – |
| Helminth eggs (Ova/10g) | $<1.0^{a}$ | $<1.0^{a}$ | – | – |

Note: Different letters on a row show significant differences at $p < 0.05$. ECN-QAS = European Compost Network-Quality Assurance Scheme. Source: Ecocert [35]; Leifert [36].

### 3.2.2. Main effects of storage duration on NECo and Co

The main effect of storage duration was also significant at $p < 0.05$ for total coliform for both fertilisers (NECo and Co) as shown in Figure 2. In NECo, total coliform counts were observed only in months 1 and 2 during storage and these were not significantly different. This indicates that longer storage duration reduces coliform counts in NECo below detection limits.

In the case of Co in storage, total coliform was observed during each month of storage except in month 5 where levels were observed to be below detection limits. There was a general increase in coliform counts from 1.8 log units in month 1 to 4.1 log unit in month 2 before gradually declining to 2.2 log units in month 6 (Figure 2). The total coliform counts were significantly different between month 2 and months 1 and 6. Results indicate that longer duration of storage may lower total coliform counts of Co. Storage duration effect on *E. coli* in NECo and Co was not observed as *E. coli* was absent (below detection limit).

### 3.2.3. Interaction effects between storage temperature and storage duration on NECo and Co

The interaction effect between storage temperature and storage duration on total coliform was significant at $p < 0.05$ for NECo but not statistically significant for Co (table not shown). The interaction effect on *E. coli* was also not significant in both NECo and Co. Findings show that NECo recorded highest levels of total coliform during the month 2 of storage under 25°C temperature conditions (Table 2). At longer durations of storage beyond month 2, coliform levels were below detection limits (absent) irrespective of the temperature conditions of storage.

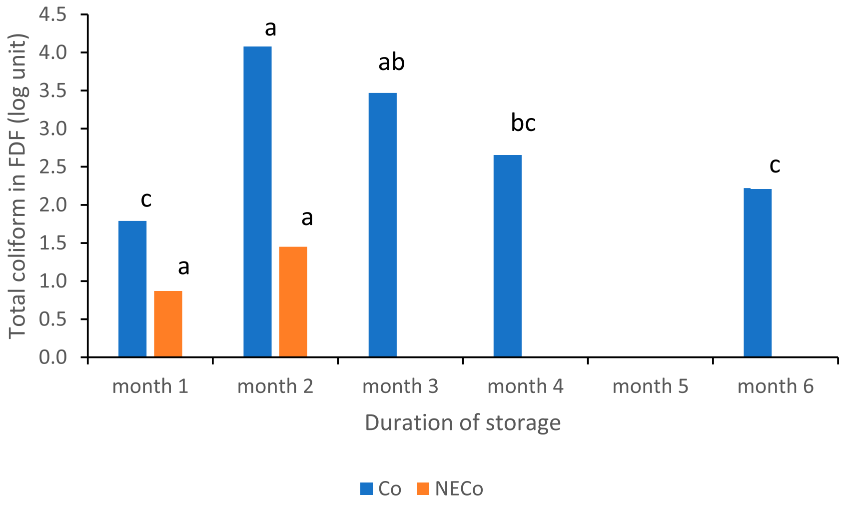


**Figure 2.** Main effect of storage duration on total coliform count in NECo and Co.

### 3.3. Effects of storage temperature and storage duration on nutrient and heavy metals characteristics in stored faecal derived fertilisers

#### 3.3.1. Main effects of storage temperature on fertiliser pH, EC, and nutrient characteristics

The pH, total N, and $NH_4$-N in both stored NECo and Co under the different storage temperature conditions were not significant at $p < 0.05$ in Table 3. Storage temperature affected only EC, organic carbon (OC) and total K of NECo fertilisers. Decreasing storage temperatures led to an increase in EC of NECo (Table 3).

The results also show that in Co fertiliser, more $NO_3$ concentration was conserved at ambient temperatures than at lower temperatures (Table 3). While total P was highest in Co at the lowest temperature of 5°C. Total P had increased in all storage temperatures for all FDFS from the initial 1.8% in Table 1. Organic carbon (OC) did not differ between 5°C and 25°C storage temperatures.

#### 3.3.2. Main effects of storage duration on fertiliser pH, EC, and nutrient characteristics

***3.3.2.1. Co.*** The main effect of storage duration on Co was not significant for total N and $NO_3$ (Table 4). The pH of Co after 6 months of storage duration did not change from the initial pH of 8.7 prior to storage. Though the pH lowered during months 1 and 2. This similar pattern was also observed in the EC of Co shown in Table 4. Total P and total K concentrations increased with storage duration from an initial 1.8% and 1.8% respectively, in Table 1 to 3.0% and 3.2% respectively after 6 months of storage.

***3.3.2.1 NECo.*** Similarly, to Co, the main effect of duration on NECo was not significant for total N and $NO_3$ (Table 4). However, pH of NECo increased in storage from 6.1 to 7.5 after 6 months duration. The highest pH of 7.7 was observed in month 3 of storage. The EC of NECo also increased with storage duration.

#### 3.3.3. Interaction effect between storage temperature and duration on fertiliser nutrient characteristics

***3.3.3.1. Co.*** The interaction effect between storage temperature and duration of storage on Co characteristics were not significant for the major nutrients (total N, total P and K). The interaction effect was, however, significant at $p < 0.05$ for OC, EC, $NH_4$-N, $NO_3$-N, and pH as described in Table 5. $NH_4$-N of stored Co under 5°C and 25°C temperature conditions did not generally change with increasing duration of storage. The organic carbon (OC) concentrations in month 1 of storage were higher under ambient conditions (18.8%) than in the lower storage temperatures. However,

**Table 2.** Interaction effect between storage duration and temperature on total coliform levels of NECo (log units/g).

| | Duration | | | | | |
|---|---|---|---|---|---|---|
| Storage temperature | Month 1 | Month 2 | Month 3 | Month 4 | Month 5 | Month 6 |
| 5°C | $1.2^b$ | $1.1^b$ | $0.0^b$ | $0.0^b$ | $0.0^b$ | $0.0^b$ |
| 25°C | $0.8^b$ | $3.2^a$ | $0.0^b$ | $0.0^b$ | $0.0^b$ | $0.0^b$ |
| Ambient | $0.6^b$ | $0.0^b$ | $0.0^b$ | $0.0^b$ | $0.0^b$ | $0.0^b$ |

Note: Different letters in a column show significant differences at $p < 0.05$.

**Table 3.** Main temperature effect on stored faecal derived fertiliser pH, EC and nutrient characteristics.

| Temperature conditions | pH (1:5) | EC (1:10) (mS/cm) | Organic carbon (%) | Total N (%) | $NH_4$-N (mg/kg) | $NO_3$-N (mg/kg) | Total P (%) | Total K (%) |
|---|---|---|---|---|---|---|---|---|
| NECo | | | | | | | | |
| 5°C | $7.5^{a}$ | $14.7^{a}$ | $27.8^{a}$ | $2.5^{a}$ | $1038^{a}$ | $187^{a}$ | $4.7^{a}$ | $2.0^{c}$ |
| 25°C | $7.5^{a}$ | $14.1^{b}$ | $27.9^{a}$ | $2.6^{a}$ | $1026^{a}$ | $213^{a}$ | $3.5^{b}$ | $2.6^{a}$ |
| Ambient | $7.5^{a}$ | $14.2^{b}$ | $26.8^{b}$ | $2.2^{a}$ | $975^{a}$ | $172^{a}$ | $3.8^{ab}$ | $2.2^{b}$ |
| Co | | | | | | | | |
| 5°C | $8.7^{a}$ | $5.4^{a}$ | $27.0^{a}$ | $1.2^{a}$ | $32^{a}$ | $115^{b}$ | $3.6^{a}$ | $3.1^{a}$ |
| 25°C | $8.7^{a}$ | $5.4^{a}$ | $26.9^{a}$ | $1.1^{a}$ | $33^{a}$ | $115^{b}$ | $2.2^{b}$ | $2.9^{a}$ |
| Ambient | $8.7^{a}$ | $5.4^{a}$ | $26.6^{a}$ | $1.2^{a}$ | $42^{a}$ | $135^{a}$ | $2.6^{b}$ | $2.8^{a}$ |

Note: Different letters in a column show significant differences at $p < 0.05$ for each FDF.

**Table 4.** Main effect of storage duration on pH, EC, and nutrient characteristics of FDFs.

| Storage duration | pH (1:5) | EC (1:10) (mS/cm) | Organic carbon (%) | Total N (%) | $NH_4$-N (mg/kg) | $NO_3$-N (mg/kg) | Total P (%) | Total K (%) |
|---|---|---|---|---|---|---|---|---|
| NECo | | | | | | | | |
| Month 1 | $7.3^{d}$ | $15.6^{a}$ | $17.1^{c}$ | $2.5^{a}$ | $994^{ab}$ | $163^{bc}$ | $1.6^{b}$ | $2.0^{c}$ |
| Month 2 | $7.5^{c}$ | $14.6^{b}$ | $18.4^{c}$ | $2.4^{a}$ | $1067^{ab}$ | $166^{bc}$ | $4.3^{a}$ | $2.1^{bc}$ |
| Month 3 | $7.7^{a}$ | $13.1^{c}$ | $31.3^{b}$ | $2.4^{a}$ | $1187^{a}$ | $105^{c}$ | $4.6^{a}$ | $2.2^{abc}$ |
| Month 4 | $7.6^{bc}$ | $14.4^{b}$ | $32.6^{ab}$ | $2.4^{a}$ | $1262^{a}$ | $117^{c}$ | $4.7^{a}$ | $2.5^{a}$ |
| Month 5 | $7.6^{b}$ | $13.9^{bc}$ | $32.8^{a}$ | $2.4^{a}$ | $788^{b}$ | $242^{ab}$ | $4.8^{a}$ | $2.4^{ab}$ |
| Month 6 | $7.5^{c}$ | $14.2^{b}$ | $32.5^{ab}$ | $2.6^{a}$ | $782^{b}$ | $351^{a}$ | $4.0^{ab}$ | $2.4^{ab}$ |
| Co | | | | | | | | |
| Month 1 | $8.4^{c}$ | $5.9^{a}$ | $17.1^{c}$ | $1.2^{a}$ | $27^{b}$ | $139^{a}$ | $2.0^{b}$ | $2.3^{b}$ |
| Month 2 | $8.6^{b}$ | $5.7^{ab}$ | $16.5^{c}$ | $1.2^{a}$ | $24^{b}$ | $125^{a}$ | $2.7^{ab}$ | $2.8^{ab}$ |
| Month 3 | $8.8^{a}$ | $5.0^{d}$ | $31.0^{b}$ | $1.2^{a}$ | $31^{b}$ | $121^{a}$ | $2.8^{ab}$ | $3.0^{a}$ |
| Month 4 | $8.8^{a}$ | $5.5^{bc}$ | $32.0^{ab}$ | $1.2^{a}$ | $46^{ab}$ | $124^{a}$ | $2.9^{ab}$ | $3.2^{a}$ |
| Month 5 | $8.7^{a}$ | $5.0^{d}$ | $32.5^{a}$ | $1.1^{a}$ | $29^{b}$ | $109^{a}$ | $3.3^{a}$ | $3.2^{a}$ |
| Month 6 | $8.7^{a}$ | $5.3^{cd}$ | $32.0^{ab}$ | $1.0^{a}$ | $59^{a}$ | $111^{a}$ | $3.0^{ab}$ | $3.2^{a}$ |

Note: Different letters in a column show significant differences at $p < 0.05$ for each FDF.

beyond month 2 of storage there was no apparent significant differences in the OC between the different storage temperatures for the duration of the storage period.

*3.3.3.2. NECo.* The interaction effect on NECo was not significant for OC and the main nutrient (total N, total K, and total P) characteristics just was observed for Co. The characteristics that were significant are shown in Table 6. $NH_4$-N concentrations decreased with increasing duration of storage for NECo stored at lower temperatures (5°C and 25°C). Under ambient storage conditions, $NH_4$-N concentrations increased with increasing storage duration (Table 6). Results also show that $NO_3$-N concentrations increased generally in NECo stored under the different temperature conditions after 6 months.

**Table 5.** Interaction effect between storage temperature and duration on Co nutrient characteristics.

| | Duration | | | | | |
|---|---|---|---|---|---|---|
| Storage temperature | Month 1 | Month 2 | Month 3 | Month 4 | Month 5 | Month 6 |
| | | | $NH_4$-N (mg/kg) | | | |
| 5°C | $42.5^{bc}$ | $21.2^{bc}$ | $22.3^{bc}$ | $20.9^{bc}$ | $36.0^{bc}$ | $50.4^{abc}$ |
| 25°C | $26.6^{bc}$ | $28.8^{bc}$ | $32.4^{bc}$ | $50.2^{abc}$ | $29.2^{bc}$ | $32.0^{bc}$ |
| Ambient | $11.5^{c}$ | $20.9^{bc}$ | $36.7^{bc}$ | $67.0^{ab}$ | $22.0^{bc}$ | $94.0^{a}$ |
| | | | $NO_3$-N (mg/kg) | | | |
| 5°C | $101.1^{b}$ | $120.4^{ab}$ | $113.1^{ab}$ | $121.0^{ab}$ | $124.6^{ab}$ | $106.7^{ab}$ |
| 25°C | $132.2^{ab}$ | $143.1^{ab}$ | $127.1^{ab}$ | $121.8^{ab}$ | $67.8^{b}$ | $99.1^{b}$ |
| Ambient | $183.1^{a}$ | $112.0^{ab}$ | $123.8^{ab}$ | $128.8^{ab}$ | $135.0^{ab}$ | $126.8^{ab}$ |
| | | | pH (1:5) | | | |
| 5°C | $8.4^{ef}$ | $8.5^{cdef}$ | $8.8^{ab}$ | $8.8^{ab}$ | $8.8^{ab}$ | $8.7^{abcd}$ |
| 25°C | $8.5^{def}$ | $8.6^{bcdef}$ | $8.7^{abcd}$ | $8.9^{a}$ | $8.7^{abcde}$ | $8.8^{abc}$ |
| Ambient | $8.3^{f}$ | $8.7^{abcde}$ | $8.9^{a}$ | $8.8^{ab}$ | $8.7^{abcd}$ | $8.7^{abcd}$ |
| | | | EC (1:10) (mS/cm) | | | |
| 5°C | $5.6^{abc}$ | $5.7^{ab}$ | $5.0^{cde}$ | $5.5^{abcd}$ | $4.8^{de}$ | $5.7^{ab}$ |
| 25°C | $5.9^{ab}$ | $5.7^{ab}$ | $5.3^{bcde}$ | $5.4^{abcde}$ | $4.9^{cde}$ | $5.4^{bcde}$ |
| Ambient | $6.1^{a}$ | $5.8^{ab}$ | $4.7^{e}$ | $5.5^{abcd}$ | $5.3^{bcde}$ | $4.9^{cde}$ |
| | | | OC (%) | | | |
| 5°C | $15.7^{d}$ | $16.3^{cd}$ | $31.6^{ab}$ | $33.1^{a}$ | $32.7^{ab}$ | $32.3^{ab}$ |
| 25°C | $16.8^{cd}$ | $16.7^{cd}$ | $31.3^{ab}$ | $31.7^{a}$ | $32.6^{ab}$ | $32.6^{ab}$ |
| Ambient | $18.8^{c}$ | $16.6^{cd}$ | $30.0^{b}$ | $31.2^{ab}$ | $32.1^{ab}$ | $30.8^{ab}$ |

Note: Different letters in a column show significant differences at $p < 0.05$ for each parameter.

**Table 6.** Interaction effect between storage temperature and duration on NECo nutrient characteristics.

| Storage temperature | Duration<br>Month 1 | Month 2 | Month 3 | Month 4 | Month 5 | Month 6 |
|---|---|---|---|---|---|---|
| | | | $NH_4$-N (mg/kg) | | | |
| 5°C | $1138^{abc}$ | $994^{abc}$ | $1254^{ab}$ | $1270^{ab}$ | $748^{abc}$ | $827^{abc}$ |
| 25°C | $1369^{a}$ | $1284^{ab}$ | $993^{abc}$ | $1259^{ab}$ | $636^{bc}$ | $612^{bc}$ |
| Ambient | $475^{c}$ | $922^{abc}$ | $1314^{ab}$ | $1256^{ab}$ | $978^{abc}$ | $907^{abc}$ |
| | | | $NO_3$-N (mg/kg) | | | |
| 5°C | $219^{abc}$ | $160^{bc}$ | $118^{bc}$ | $147^{bc}$ | $143^{bc}$ | $334^{ab}$ |
| 25°C | $102^{bc}$ | $104^{bc}$ | $48^{c}$ | $110^{bc}$ | $456^{a}$ | $459^{a}$ |
| Ambient | $166^{bc}$ | $235^{abc}$ | $150^{bc}$ | $94^{bc}$ | $127^{bc}$ | $262^{abc}$ |
| | | | pH (1:5) | | | |
| 5°C | $7.3^{hi}$ | $7.4^{ghi}$ | $7.7^{a}$ | $7.7^{abc}$ | $7.7^{abc}$ | $7.5^{defg}$ |
| 25°C | $7.4^{fgh}$ | $7.5^{defg}$ | $7.7^{abc}$ | $7.6^{bcdef}$ | $7.6^{abcd}$ | $7.5^{defg}$ |
| Ambient | $7.3^{i}$ | $7.6^{abcde}$ | $7.7^{ab}$ | $7.4^{efg}$ | $7.6^{abcde}$ | $7.5^{cdefg}$ |
| | | | EC (1:10) (mS/cm) | | | |
| 5°C | $16.0^{a}$ | $15.0^{abc}$ | $13.5^{bcd}$ | $14.1^{abcd}$ | $13.7^{bcd}$ | $15.8^{a}$ |
| 25°C | $15.3^{ab}$ | $14.2^{abcd}$ | $13.0^{d}$ | $14.5^{abcd}$ | $13.8^{bcd}$ | $13.7^{bcd}$ |
| Ambient | $15.5^{ab}$ | $14.7^{abcd}$ | $12.9^{d}$ | $14.6^{abcd}$ | $14.2^{abcd}$ | $13.2^{cd}$ |

Note: Different letters in a column show significant differences at $p < 0.05$ for each parameter.

After 6 months of storage, the Cd and Cr levels in both Co and NECo did not meet the ECN-QAS standards as indicated in Table 1 because their concentrations were higher. However, Cu, Zn, Pb, and Hg levels conformed to the ECN-QAS standards.

## 4. Discussion

### 4.1. Faecal derived fertiliser characteristics before and after enrichment

Aerobic co-composting of DFS and uncooked FW into FDF has demonstrated its effectiveness to inactivate completely or reduce faecal pathogens to undetectable levels while making nutrients and organic matter safe for crop growth [14,37]. According to Williams [26], *E. coli* is used as an indicator for the microbiological quality of sludge-derived products destined for agricultural recycling and of the efficacy of the sludge treatment processes to demonstrate the presence of faecal and pathogenic bacteria and their removal. The results from this study showed the absence of *E. coli* or below detectable limit (<1 CFU/g) in the FDFs (NECo and Co) produced though total coliforms were still present. The presence of total coliforms does not necessarily confirm faecal contamination as some total coliforms could be of environmental origins. The quality of NECo and Co did not meet the ECN-QAS or Ecocert standards for selected heavy metals [36]. The higher levels of Cd and Cr might have been from the raw FS from pit latrines and septic tanks used as feedstock. Users of these latrines dump rubbish/solid wastes which might have contained batteries, consumer electronics, newspapers, paints into these latrines thus contributing to elevated levels of the Cd and Cr in the sludge.

This is further supported by a study by Oghenerobor et al. [38] who stated various sources of heavy metals that find their way into faecal sludge, and they included man-made sources like paint chips, used motor oils, batteries, ceramics, consumer electronics and natural sources like soil erosion. One of the ways to ensure FDFs meet the standards for heavy metals is to alter the feedstock mixing ratio at the beginning of co-composting. Enriching Co with mineral N fertiliser sources into NECo further lowered the pH of NECo resulting in further sanitisation of NECo by reducing the coliform numbers below detectable limits. This phenomenon was also observed by Adamtey et al. [23] in a similar study involving enrichment of FDF with various mineral nitrogen sources. In their study, Adamtey et al. [23] found out that, after enrichment, the pH of compost increased in the urea-based products while a decrease in pH was observed in the ammonium sulphate-based products. A similar observation was made in this study where the pH of Co reduced from 8.7 to 6.1 after the ammonium nitrate-based enrichment to form NECo. The lowering of pH in NECo could be attributed to nitrification, a set of processes that result in bacterially-mediated oxidation of ammoniacal nitrogen to nitrate with the associated release of protons [39].

Organic carbon (OC) was observed to be higher in NECo than in Co after enrichment in this study. This was contrary to expectations, since the enrichment was targeted at increasing N concentration and thus expected not to affect the OC. Some heavy metal levels of NECo after enrichment like Pb, Cr, Cd, and As were reduced while Fe, Mn, and Se levels increased. The reduction in heavy metal levels may be attributed to biological processes by the microbial community in NECo. The increase in Fe, Mn, and Se concentrations may have been due to the mineral fertiliser used for the enrichment. Similar findings were observed by John et al. [40] when they utilised urea enrichment during the composting of cattle dung and poultry manure. They found increased concentration of Fe, Zn, Cu, and V in the enriched compost than in the non-enriched while Ni and Pb concentration reduced in the enriched compost compared with the non-enriched.

### 4.2. Effects of storage temperature and storage duration on indigenous pathogen counts in stored faecal derived fertilisers

A key condition for maintaining quality of a fertiliser in storage is ensuring its integrity and efficacy in terms of its chemical composition and physical structure is preserved [19]. In this study, all the FDFs (NECo and Co) placed in storage recorded *E. coli* below detectable limits. This simply means that there was no indigenous re-growth as the conditions of storage did not offer favourable environment for *E. coli*. Additionally, it also mean that the co-composting treatment effectively and completely deactivated all viable *E. coli* prior to storage. This finding confirms the efficacy of co-composting as a viable treatment for producing safe NECo and Co for sub-Saharan Africa. This is similar to earlier studies which found high temperatures to be responsible for the rapid inactivation of *E. coli* in co-composting piles [37,41].

Total coliforms were present at different levels, and this was affected by both storage temperature conditions and storage duration. Though total coliforms are commonly found in the environment and as such are not directly associated with faecal contamination, they were found to be significantly lower in the NECo because of the enrichment process. Lower counts of total coliforms were observed at ambient temperature conditions in both NECo and Co at longer storage durations. This means the relatively higher temperature of ambient conditions accelerated the die-off of the total coliforms. This shows that temperature is an important factor affecting the survival of total coliforms [15]. Chen et al. [42] observed similar findings that *E. coli* and *S. enterica* survived for longer periods in composts at 5°C than at 22°C and greenhouse conditions. Wang et al. [43] also reported similar findings for a non-O157 Shiga toxin-producing *E. coli* which survived better at 4°C than 22°C for 125 days in dairy compost. The main effect of storage duration on total coliform levels saw presence in only months 1 and 2 for NECo and in all months of storage for Co except at month 5. This implies that longer durations of storage suppressed coliforms level in both NECo and Co possibly due to competition for resources/nutrients running out or competition from other microorganisms. Contrary to our findings on coliforms, Sidhu et al. [25] found out that long-term storage of the compost is not recommended as the inactivation rate of *Salmonella* decreased significantly with longer storage times, most likely due to the decline in indigenous microflora over time.

The interaction effect between storage temperature and storage duration was not statistically significant for Co. However, in NECo saw higher coliform counts at lower storage temperatures and at shorter storage duration. Implying that, in NECo, lower storage temperatures and shorter duration enhanced coliform levels. This study shows that it is generally advantageous to the store NECo under ambient temperature conditions for at least 2 months. This is because ambient conditions require lower energy to store the fertiliser. This makes it a less costly option for small and medium-scale enterprises (SMEs) who operate FS treatment plants to store FDFs.

### 4.3. Effects of storage temperature and storage duration on physico-chemical characteristics of stored faecal derived fertilisers

All factors affected the physico-characteristics differently. The duration of storage and temperature condition did not have any effect on total N levels of the FDFs (NECo and Co). The main effect of temperature conditions did not influence distinct differences in physico-chemical parameters in both NECo and Co. The effect of storage duration saw an increase in $N0_3$-N in NECo with increasing duration of storage. This is linked to nitrification process which converts ammonium to nitrite and then to nitrate [23]. This similar trend of nitrification was also observed for the interaction effect of storage duration and temperature on the FDF where higher nitrification occurred at low storage temperatures especially at 25°C. This was occurring in NECo fertiliser after 6 months of storage and at lower storage temperatures. Nitrification is important because plants mostly take up N from the soil in the form of $NO_3$-N. The $NH_4$-N and $NO_3$-N concentrations of Co did not significantly change over the storage duration and temperature indicating a quite minimal N mineralisation of Co during storage.

Total P and K levels were not affected by interaction between temperature and duration of storage on NECo or Co. This agrees with the findings of Pawłowska et al. [19] that 1 year storage under different conditions did not significantly influence concentrations of total P and total K. The findings from this study reveal that higher nutrient content and conservation is likely with longer duration of storage irrespective of the storage temperatures. The heavy metal levels of the FDFs after the storage period of 6 months did not meet the ECN-QAS or Ecocert standards. This may imply that the storage of NECo and Co under different temperature conditions and storage did not significantly affect heavy metal concentrations. These elements find their way from the feedstock such as food waste or FS through contamination at the point of generation/containment. There must be mechanisms put in place along the sanitation value chain to minimise the contamination with potential sources of heavy metals.

In this study, we found that the interaction effect of all factors (storage temperature and duration) on pH and EC did not exhibit a clear increasing or decreasing trend. While pH was on a slight increasing trend with longer duration of storage in Co, there was however not a clear increasing or decreasing trend observed for NECo with duration of storage. This trend with pH and EC observed was similar to the findings of Kleawklaharn and Iwai [44] who found out that, the chemical properties of the vermicompost were changing differently, and yet there was no clear trend in the changes of pH, EC, total potash and magnesium during the different duration (0, 1, and 3 months) of storage. The enrichment process [23] may have increased the EC of the NECo just like the enrichment also affecting the pH of the FDF by lowering the pH of the NECo [39]. Organic carbon (OC) concentration doubled from month 2 to month 3 onwards in NECo and Co for the storage duration. This was also similar for the interaction effect between storage temperature and duration in Co. No reason known could be attributed to these findings.

## 5. Conclusion

This study concludes that extrinsic abiotic factors such as storage duration and temperature conditions affected indigenous pathogen and physico-chemical properties of FDFs (NECo and Co). Findings from this study showed that storage temperature and duration did not affect or influence indigenous *E. coli* re-growth in stored NECo or Co. However, total coliform levels were affected by all factors which do not confirm faecal contamination. Therefore, the absence of *E. coli* is an important finding for the FDF industry and the public as it shows how microbiologically safe NECo and Co are even after storage. Storage temperature and duration did not have any effect on total N in NECo or Co. Nutrient retention is more with longer duration of storage irrespective of storage temperature in Co. However, losses of $NH_4$-N occur in NECo with longer duration and at lower temperatures. The importance of this findings is that longer storage periods do influence nutrient losses in stored NECo but not in stored Co. Heavy metal concentrations in FDF especially Cd, Pb, and Cr did not meet the criteria set by ECN-QAS and Ecocert standards prior to storage and even after storage.

It is worth noting that the effect of moisture as an additional abiotic factor was not considered in this study. Varying moisture conditions may play a critical role for pathogen re-growth in FDF. In this study, we considered only the production moisture content of FDF. Future studies are therefore recommended to investigate the effect of moisture content on pathogen and nutrient characteristics of stored FDF.

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## Data availability statement

Data supporting this study are openly available from CORD https://doi.org/10.17862/cranfield.rd.24570343.

## ORCID

*Ruben Sakrabani* http://orcid.org/0000-0003-1271-7044
*Sean Tyrrel* http://orcid.org/0000-0002-6352-9839

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