TRANSACTIONS ON SCIENCE AND TECHNOLOGY

Effects of sterilization, humic acid and indigenous microbial formulation on physicochemical properties and macro-micronutrients of dairy farm effluent compost

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ABSTRACT Biostimulant application can enhance compost's physicochemical properties, nutrient content and performance. Dairy farm effluent compost (DFEC) is a promising planting medium for leafy vegetable production. However, little is known about its quality after biostimulant application. The present study was carried out to evaluate the effects of humic acid (HA) and indigenous microbial formulation (IMF) on DFEC's physicochemical properties and macro-micronutrients. Sterilised (ST) or non-sterilised (NST) DFECs were added with HA, IMF or NPK 15:15:15 fertiliser, or a combination of them; there were eight amendments (A1-A8). The amended DFECs were re-used four times for Pak Choy cultivation; the plants were planted in forty-eight 18 L pots. DFEC samples were collected from each pot at the second (MR2), third (MR3) and fourth (MR4) harvesting of the Pak Choy, and the physicochemical properties (pH, EC, C/N ratio, OM, OC, WHC) and macro-micronutrients (N, P, K, Mg, Ca, Na, Mn, Fe, Zn and Cu) were evaluated. The macro-micronutrients were analysed using a CHN analyser and ICP-OES. The data were analysed by performing three-way ANOVA at α = 0.05 using SAS. ST-DFEC had higher pH, EC, C/N ratio, WHC, and macro-micronutrients (P, K, Ca, Na, Mn, Fe, and Cu) but lower OM and OC. HA, IMF or HA+IMF application did not significantly affect the DFEC's physicochemical properties and macro-micronutrients. The effects were significant only after NPK addition (A5-A8). HA+IMF+NPK (A8) was the best amendment to improve P, K, Ca, Na, Mn, Fe, and Cu levels. Nutrient content was better in the second and fourth re-usage of the compost. The data indicated that DFEC's agronomic quality could be improved by sterilization, NPK+HA+IMF addition or NPK and at least one of the biostimulants, re-usage with NPK and biostimulant applications, or a combination of those amendments.

KEYWORDS: Dairy farm effluent compost; Humic acid; Indigenous microbes; Pak Choy; Compost amendments.
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INTRODUCTION

Compost industry contributes to the improvement of agricultural waste management and the development of farming industry. Farmers use composts as a stand-alone substrate, an organic top dress, or an incorporated soil amendment (Fitzpatrick, 1998). In urban areas with limited land and soil supply, compost as a stand-alone substrate is a practical alternative for crop production. In a rooftop garden, soil could be too heavy to use, and thus, the media are usually a mixture of organic matter (OM), such as compost, and lightweight aggregate or shale (Walters & Midden, 2018). For that purpose, the compost needs to be appropriately readied. Non-soil medium needs to have optimum physical-chemical properties and be supplied with optimal storage of nutrients and water for better crop yield (Raviv *et al*., 2008).

Dairy farm effluent compost (DFEC) is a promising planting medium for urban farming. It can easily be obtained from the dairy industry. In Sabah, Malaysia, more than 500 tons of DFEC are

produced monthly (Radius, 2024). DFEC supports well leafy vegetable (Pak Choy or Brassicas) production as a stand-alone substrate (Maludin *et al*., 2019). With such potential, it can be a substitute for soil to increase local Pak Choy production and mitigate leafy vegetable import. In Malaysia, USD 162 million worth of leafy vegetables, including Brassicas, were imported in 2022 (TrendEconomy, 2024). The challenge is the death rate of Pak Choy planted on DFEC is high at the first to second use (Maludin *et al*., 2019). Hence, as a stand-alone substrate, DFEC requires an amendment to improve its condition to increase crop yield right at first use.

Various materials are used in the agricultural industry as biostimulants to improve soil or media conditions. Humic acid (HA) is among the popular materials because of its effectiveness. For example, HA addition can improve spinach fresh yield from 11 g to 25 g per plant (Turan *et al*., 2022). HA improves soil and compost's physicochemical properties and enhances their structural stability (Yang *et al*., 2021). It promotes soil particle aggregation and improves soil porosity and water infiltration (Xu *et al*., 2022), which is essential for improving clay soil conditions. It has a water-attracting hydrophilic part, which increases soil, especially sandy soil, water-holding capacity (WHC) (Yang *et al*., 2021; Li *et al*., 2023). HA helps stabilise the OM in compost, leading to the formation of stable humus (Lehmann & Kleber, 2015), meaning it decreases nutrient leaching. It can also act as a natural buffer and stabilise soil pH (Khaled & Fawy, 2011). It increases the soil's cation exchange capacity (CEC) and allows it to retain essential cations, such as K, Ca, and Mg (Liu *et al*., 2021). Also, it can form complexes with micronutrients, such as Fe, Zn, and Mn, and prevents them from precipitating (Garcia *et al*., 2016). HA is a natural chelator that facilitates micronutrient chelation and retention in soil (Sible *et al*., 2021). From a plant perspective, HA enhances soil fertility (Yang *et al*., 2021) and creates a favourable environment for growth (Khaled & Fawy, 2011), root penetration (Xu *et al*., 2022), cation retention (Liu *et al*., 2021), and better micronutrient (e.g., Fe, Zn and Mn) and macronutrient (e.g., N, P, and K) uptake (Canellas *et al*., 2002; Garcia *et al*., 2016; Sible *et al*., 2021). Overall, HA addition improves soil and compost's agronomical conditions, longevity, recyclability, and effectiveness for crop production.

Microbial formulation (MF), such as indigenous MF (IMF), is another popular soil or media biostimulant. The formulation often comprises plant growth-promoting microorganisms, such as bacteria (*Bacillus* strains) and fungi (*Trichoderma*). Microbial formulation (*Trichoderma*-based biostimulant) can improve crop produce, such as marketable lettuce yield in non-fertilised soil from 400 g to 500 g per plant (Fiorentino *et al*., 2018). IM addition shifts soil's microbial community structure, enhancing beneficial microbial populations while suppressing pathogenic ones (Berendsen *et al*., 2012). It increases beneficial bacterial groups, such as Gammaproteobacteria and Acidobacteria (Wang *et al*., 2018). It promotes a stable aggregate formation, as the beneficial microbes produce extracellular polysaccharides, which help bind soil particles together, thus improving soil bulk density and porosity, water infiltration, soil structure and aeration (Bachar *et al*., 2010; Ni *et al*., 2024). IM application can also contribute to soil pH buffering capacity (Nannipieri *et al*., 2003). It increases enzyme activities and plays crucial roles in nutrient cycling and OM decomposition (Wang *et al*., 2018). It can increase macro-micronutrient availability, such as N, P, K, Fe and Zn (Ramesh *et al*., 2014), Ca and Mg (Trabelsi & Mhamdi, 2013), and copper (Cu) and Mn (Singh *et al*., 2022), for plant uptake. Generally, beneficial microbe addition maintains a balanced microbial ecosystem, enhances OM decomposition, fixates nutrients, sustains optimal soil pH levels, and maintains nutrient cycling (Nannipieri *et al*., 2003; Wang *et al*., 2018). Ultimately, it enhances soil health and fertility, improves root formation and penetration, increases plant nutrient uptake, and supports better plant growth.

When HA and IMF are applied together, the synergic effects on soil or media conditions and vegetable production are often amplified. HA and microbial inoculant (*Pseudomonas fluorescens*) can improve, for instance, cabbage fresh yield from 25.8 to 37.9 t/ha (Verma *et al*., 2017). Cabbage yield under HA or *P. fluorescens* addition alone is only 33.5 and 32.0 t/ha, respectively. HA stimulates microbial activity, such as microorganism nutrient-solubilising activity, increases OM decomposition and mineralisation, and enhances nutrient release (Plaza *et al*., 2005). HA+MF application improves soil structure and mitigates nutrient leaching, lessening soil degradation and enhancing nutrient retention capacity (Chen *et al*., 2019). HA+MF also improves macro-micronutrient availability, such as N, P, K, Fe, Zn, and Ca (Schoebitz *et al*., 2016). HA creates a favourable environment for better soil microbial growth and activity (Khaled & Fawy, 2011), while microbes enhance physical stability. The combination significantly improves overall soil and compost agronomic quality and nutrient recycling (Pandit *et al*., 2023).

Sterilization is another alternative for biostimulant application to improve soil or media conditions. It could alter soil microbial and physicochemical properties, thus improving crop yields (Tian *et al*., 2009; Li *et al*., 2019a). Its effects, however, will depend on the methods used, as it could eliminate both plant pathogenic (Li *et al*., 2019a) and beneficial microbes in soils (Ochieno, 2022). Nevertheless, it provides a chance for artificial re-introduction of specific beneficial microbial formulations in soils, which is better because it allows targeting a particular soil and crop performance (Ochieno, 2022).

Combining biostimulant applications and compost sterilization can be an effective approach to improve DFEC quality. However, to date, little is known about changes in physicochemical properties and nutrient content of DFEC after biostimulant (HA, IMF, or HA+IMF) addition and sterilization (e.g., heat treatment), especially in re-usage conditions, whether they are improving or deteriorating. Hence, the present research was carried out to investigate the physicochemical properties and macro-micronutrient changes in DFEC after HA, IMF, HA+IMF, and sterilization treatments. This study will provide information that can guide farmers in using HA, IMF, and sterilization as DFEC amendments for commercial leafy vegetable production and fully tap the compost potential. The latter aligns with Malaysia's focus on establishing sustainable crop and vegetable farming (Tiraieyari *et al*., 2014) using agro-waste-based composts (Murad *et al*., 2008).

METHODOLOGY

Study Site and General Experimental Set-Up

The study was carried out in an open-air rain shelter (UV-plastic roof and insect-proof wall) at the Faculty of Sustainable Agriculture, Universiti Malaysia Sabah, Sandakan, Sabah. The shelter's average air temperature and humidity from 8 AM to 6 PM were 25 °C–38 °C and 50%–91%, respectively. The DFEC was obtained from the compost stock in the faculty; it was a three-week-old DFEC (after-production). Forty-eight 16 L compost packs (3 kg/pack) were prepared, and half were sterilised (autoclaved). Each pack was placed in 18 L pots (48 pots) and subjected to the amendments (Table 1). All pots with the compost were used four times (reused) for Curly Dwarf Pak Choy (CDP) cultivation (Figure 1; three Pak Choy were cultivated per pot). Compost samples were collected from each pot before the first planting and after each harvesting of the CDP. During the experiment, the CDPs were watered daily with 500 mL of distilled water at 8 AM and again at 5 PM. Weeds were removed by pulling with hands.

Compost Sterilization and Amendments

The sterilised DFECs (ST-DFEC) were autoclaved at 121 °C of 15 psi for 15 min; the entire process took 2 hr to finish. The DFECs were sterilised to eliminate the existing microbial population in the compost so that the IMF addition effects on the compost's physicochemical properties and macro-micronutrients were explicitly investigated. Once prepared, the ST-DFEC and non-sterilised DFEC (NST-DFEC) were appropriately kept in the rain shelter until used. The HA and IMF applications were carried out based on the set-up in Table 1 following a completely randomised design. HA of 1.5 g/L was added just before and 20 days after sowing (DAS) (Raheem, 2018). IMF of 818 L/ha (81.8 mL/m^2) was added one week before sowing (Zuraihah *et al.*, 2012). NPK 15:15:15 of 150 kg/ha (15 g/m^2) was applied at 20 DAS and 30 DAS based on the recommendation by MARDI (MARDI, 2005). The HA, IMF and NPK 15:15:15 concentrations were standardised based on the pot surface area (0.71 m^2/pot).

Table 1. HA and IMF amendments of the DFEC.

Amendments	Components	
	Non-sterilised (NST)	Sterilised (ST)
A1 (control)	DFEC	DFEC
A2	DFEC + IMF	DFEC + IMF
A3	DFEC + HA	DFEC + HA
A4	DFEC + IMF + HA	DFEC + IMF + HA
A5	DFEC + NPK	DFEC + NPK
A6	DFEC + NPK + IMF	DFEC + NPK + IMF
A7	DFEC + NPK + HA	DFEC + NPK + HA
A8	DFEC + NPK + IMF + HA	DFEC + NPK + IMF + HA

Figure 1. CDP cultivation on HA and IMF amended DFEC in pot system.

Compost Sample Collection, Preparation and Physicochemical Property Assessment

A 100 g of the top 0–10 cm DFEC in each pot was collected, air-dried at room temperature, ground manually using mortar and pestle, filtered through a 2 mm sieve, and kept in capped cups

for future analysis. Briefly, 5 g of sieved samples were tested in every evaluation of pH, EC, OM, organic carbon (OC), C/N ratio, and WHC before treatments. The samples were collected, processed and tested again after the second (second media re-usage: MR2), third (MR3) and fourth (MR4) harvesting of the CDPs.

Determination of OM and OC was carried out using the loss of weight on the ignition method as described by Chefetz *et al*. (1996). Five grams of the sieved samples were placed in a 30 mL ashing vessel and dried in an oven at 105 °C for 4 hr. The samples were then left to cool at room temperature and weighed to the nearest 0.01 g. The ashing vessels were placed in a muffle furnace and set at 400 °C for 4 hr to ash the samples. The ashing vessels were removed from the muffle furnace and cooled in a desiccator. The samples were then weighed to the nearest 0.01 g. The percentages of the OM and OC were calculated as follows:

Percentage of OM = $(W_1 – W_2)/W_1 \times 100$

Where: W_1 was the weight of compost at 105 °C

W_2 was the weight of compost at 400 °C

Percentage of organic C was given by % OM × 0.58

pH and electrical conductivity (EC) measurements were carried out based on the method described by (Grigatti *et al*., 2012). A 1:10 suspension of the compost was prepared by adding 5 g sieved samples with 50 mL distilled water. The suspension was stirred for 30 min at 250 rpm using an orbital shaker and filtered using a Whatman Filter Paper No. 2. The filtrate's pH and EC were measured using a pH and EC tester (Trans Instruments Professional Benchtop pH meter BP3001).

Compost Macro-Micronutrient Content Assessment

The DFEC samples were analysed for nutrient content using a benchtop photometer (HI83099, Hanna Instrument) and a CHN analyser (CHN-600, LECO Corporation, St. Joseph, MI). The sampling processes were repeated after each harvesting (MR2, MR3, and MR4) of the CDPs and analysed. For the total N and C assessment using a CHN analyser, each sample was weighed at 0.1 g to the nearest 0.0001 g using an analytical balance (Model TLE3002E, Mettler Toledo), placed in tin foil, appropriately secured, and dropped into the combustion tube of the analyser. The total N value per sample was divided by 100 and multiplied by 10,000 to convert from (%) to ppm. The macro (total P, K, Ca, Mg) and micro (total Na, Mn, Fe, Cu, Zn) nutrient contents were determined using a slightly adjusted single dry ashing method described by Isaac and Johnson (1975) as follows. Individual samples were air-dried in an oven at 60 °C for 24 hr and then left to cool in a desiccator before dry ashing. For every sample, 0.5 g were placed in a 30 mL porcelain dish (crucible with lid) and dried ash inside a benchtop muffle furnace (Thermolyne F47915, Thermo Scientific) at 300 °C for 1 hr and then at 520 °C for 4–5 hr until all samples turned white. The samples were left to cool before further analysis. For digestion, the samples were placed in a fume chamber, and a few drops of distilled water were added to the samples, followed by 1 mL of concentrated HCL. The samples were then evaporated to dryness using a hot plate, added with 5 mL of 20% HNO_3 and left at room temperature for 1 hr. After digestion, the samples were filtered using Whatman Filter Paper No. 2 into a 50 mL volumetric flask. The volume of the solutions was then made to the mark by adding distilled water. The solutions were transferred into a 100 mL plastic vial before being filtered using 0.45 μM hydrophilic PTFE membrane into a 50 mL centrifuge tube for storage. Before further analysis, all solutions were diluted in a 1:30 ratio (sample: distilled water). The concentrations of P, K, Mg, Ca, Fe, Na, Mn, Fe, Cu and Zn were determined using Inductively Coupled Plasma Optical Emission Spectrometry (ICP-OES Optima 5300 DV, Perkin Elmer).

Compost Water Holding Capacity (WHC) Determination

Determination of WHC was carried out based on method described by Bernard (1963). The DFEC samples before amendments and after CDPs' harvesting (MR2, MR3, and MR4) were thoroughly air-dried by spreading them to a thin layer on a plastic sheet. The WHC testing funnel was clamped at the bottom, suspended above a graduated cylinder, lined with a Whatman Paper No. 2, and filled with 50 mL of the air-dried sample. The sample was poured gradually and homogeneously with 100 mL distilled water, stirred gently, and left to sit; this step was repeated until the sample was saturated with water. The clamp was then released to allow excess water to flow into the graduated cylinder. After dripping stopped, amount of water in the graduated cylinder was recorded. The calculation of water retained and WHC were as follows:

___ mL water retained/100 mL of sample = water added (mL) – water drained (mL).

WHC (mL/L) = 10 × (water retained/100 mL sample)

The value was multiplied by 10 to convert the 100 mL sample to L to express the WHC as the amount of water retained per litre of compost.

Data Analysis

The effects of media sterilization (MS), amendments (AMT) and media re-usage (MR) on the physicochemical properties and macro-micronutrients of DFEC were analysed by performing a three-way Analysis of Variance (ANOVA) on the data obtained. Mean separation tests were carried out according to Tukey's Studentised Range (HSD). Data normality was assessed using the Saphiro-Wilks Test. All statistical analysis was performed using SAS Version 9.4 (SAS, 2016).

RESULTS

Changes in Physicochemical Properties

Physicochemical properties of DFEC before and after treatments are shown in Tables 2 and 3, respectively. MS×AMT×MR significantly affected pH, OM and OC but not EC, C/N, and WHC of the DFEC (Table 3). MS×AMT significantly affected C/N, OM, and OC; MS×MR on C/N and OC; and AMT×MR on pH, EC, OM, OC, and C/N. WHC was affected by MS×AMT and MS×MR (Table 3). The ST-DFEC had significantly higher pH, EC, and WHC (Table 3).

Table 2. DFEC's physicochemical properties and macro-micronutrient content before treatments.

Parameters	Values
pH_{water}	7.51
EC_{water}	0.45
C/N ratio	18.80
OM (%)	57.39
OC (%)	33.18
Total N (ppm)	24500.00
P (ppm)	109.17
K (ppm)	2133.33
Ca (ppm)	25.00
Mg (ppm)	190.00
Cu (ppm)	0.93
Mn (ppm)	0.15
Fe (ppm)	12.22
Zn (ppm)	0.00

Table 3. Sterilization, HA and IMF amendments, and re-usage effects on DFEC's physicochemical properties.

Factors	pH	EC (mS/cm)	C/N	OM (%)	OC (%)	WHC (%)
Media sterilization (MS)						
NST	6.29±0.07b	0.92±0.05b	4.91±0.02a	6.78±0.12a	3.93±0.07a	6.95±0.17b
ST	6.42±0.06a	1.06±0.06a	4.94±0.03a	6.46±0.12b	3.75±0.07b	9.87±0.48a
NST vs. ST	***	*	NS	**	*	***
Amendments (AMT)						
A1 (control)	6.80±0.52a	0.21±0.04b	4.97±0.52a	6.73±0.25ab	3.91±0.15ab	8.94±0.48a
A2	6.72±0.61a	0.26±0.05b	4.99±0.52a	6.47±0.26b	3.75±0.15b	8.38±0.34a
A3	6.72±0.58a	0.19±0.04b	5.00±0.52a	5.40±0.21c	3.13±0.12c	8.83±0.39a
A4	6.74±0.59a	0.17±0.03b	4.92±0.52a	6.68±0.16ab	3.87±0.09ab	8.59±0.45a
A5	5.95±1.38b	0.44±0.09a	4.82±0.52a	6.74±0.15ab	3.91±0.09ab	8.19±0.36a
A6	5.98±1.55b	0.54±0.10a	4.97±0.52a	6.45±0.17b	3.74±0.10b	8.11±0.37a
A7	5.92±1.38b	0.39±0.09a	4.83±0.52a	7.48±0.28a	4.34±0.16a	8.25±0.41a
A8	6.00±1.34b	0.37±0.10a	4.88±0.52a	7.01±0.21ab	4.07±0.12ab	8.02±0.43a
AMT vs. AMT	***	***	*	***	***	NS
Media re-usage (MR)						
MR2	6.87±0.05a	0.75±0.05c	5.20±0.03a	6.88±0.17a	3.99±0.10a	11.72±0.44a
MR3	6.36±0.05b	1.26±0.08a	4.88±0.02b	6.45±0.20b	3.74±0.12b	7.01±0.18b
MR4	5.83±0.09c	0.97±0.08b	4.69±0.02c	6.53±0.13ab	3.79±0.07ab	6.50±0.15b
MR vs. MR	***	***	***	*	*	***
Interactions						
MS × AMT	NS	NS	**	*	*	*
MS × MR	NS	NS	*	NS	*	***
AMT × MR	**	**	NS	***	***	NS
MS × AMT × MR	***	NS	NS	***	***	NS

Data: mean ± SE. Mean values in each column followed by at least one similar letter are not significantly different at $\alpha = 0.05$ (Tukey's Test). **A1**: DFEC; **A2**: DFEC + IMF; **A3**: DFEC + HA; **A4**: DFEC + IMF + HA; **A5**: DFEC + NPK; **A6**: DFEC + NPK + IMF; **A7**: DFEC + NPK + HA; **A8**: DFEC + NPK + IMF + HA. *, **, or *** = Significant at α = 5%, 1%, or 0.1%. NS = Not-significant.

NST-DFEC had significantly higher OM and OC. Both ST- and NST-DFEC were not considerably different in C/N. Of the control and amended DFEC, A1-A4 were substantially higher in pH but lower in EC than A5-A8 (Table 3). A1-A8 were not significantly different in C/N and WHC (Table 3). Also, A1, A2, A4, A5, A6 and A8 did not differ substantially in OM and OC (Table 3). However, A3 (DFEC+HA) had significantly lower OM and OC, while A7 (DFEC+NPK+HA) had markedly higher OM and OC than the rest. Even so, NPK addition alone (A5) did not change DFEC's physicochemical properties compared to the control (A1) (Table 3). IMF addition showed a trend to suppress HA's effect on DFEC's physicochemical properties when there was NPK addition; for example, A7 (DFEC+NPK+HA) was better than or equal to A8 (DFEC+NPK+HA+IMF) in the studied parameters (Table 3). The pH, EC, C/N, OM, OC, and WHC across the DFEC re-usage declined (Table 3). The pH tended to become acidic. The EC increased markedly at the third re-usage. The OM and OC fluctuated from one to another re-usage, but overall, both were declining.

Changes in Macronutrient Content

Macronutrients of the DFEC before and after treatments are shown in Tables 2 and 4, respectively. MS×AMT×MR, MS×AMT, or MS×MR significantly affected the macronutrient content (N, P, K, Mg, and Ca) of the DFEC (Table 4). AMT×MR significantly affected the rest, but not N. ST-DFEC had substantially higher P, K, Mg and Ca but indifferent N content from NST-DFEC

(Table 4). In other words, the sterilization increased the release of macronutrients in the compost, except for N. Of the control and amended DFEC, A1-A8 did not significantly differ in total N content. A1-A4 and A5-A8 significantly differed in K and Mg, with A1-A4<A5-A8 for K and A1-A4>A5-A8 for Mg (Table 4). The pattern for P was more-or-less A8≥A4-A7≥A1-A3. For Ca, it was more-or-less A8≥A1=A3-A5=A7≥A2=A6. NPK+HA+IMF and NPK+HA also enhanced Ca availability, but IMF without HA addition (see A2 and A6) did not enhance Ca availability as they were lower than the control (A1). The N content across the DFEC re-usage was MR4>MR3>MR2, while P, Mg, and Ca were MR2=MR4>M3, and K was MR3>MR2=MR4 (Table 4). Generally, macronutrient availability decreased in a fluctuating pattern across the compost re-usage.

Table 4. Sterilization, HA and IMF amendments, and re-usage effects on DFEC's macronutrients.

Treatments	N×10 (ppm)	P (ppm)	K (ppm)	Mg (ppm)	Ca (ppm)
Media Sterilization (MS)					
NST	3531.69±709.18a	5.83±0.15b	0.34±0.02b	2.05±0.06b	9.07±0.23b
ST	3534.24±1099.82a	7.51±0.19a	0.44±0.03a	2.65±0.05a	11.55±0.26a
NST vs. ST	NS	***	***	***	***
Amendments (AMT)					
A1	3529.36±2065.68a	6.28±0.35cd	0.20±0.01b	2.45±0.13ab	10.22±0.56bc
A2	3537.67±1453.42a	6.13±0.30d	0.21±0.01b	2.43±0.15ab	9.99±0.64c
A3	3552.56±1718.19a	6.35±0.35cd	0.20±0.02b	2.43±0.12ab	10.21±0.53bc
A4	3534.48±1606.63a	6.78±0.29bc	0.25±0.02b	2.57±0.10a	10.81±0.47ab
A5	3524.70±2673.92a	6.79±0.46bc	0.54±0.04a	2.18±0.13c	10.03±0.66c
A6	3547.81±1338.86a	6.66±0.42bcd	0.57±0.04a	2.18±0.12c	9.93±0.59c
A7	3512.60±2098.61a	6.95±0.42ab	0.56±0.04a	2.20±0.12c	10.29±0.60abc
A8	3524.27±1630.16a	7.45±0.42a	0.59±0.04a	2.35±0.13bc	11.00±0.58a
AMT vs. AMT	NS	***	***	***	***
Media Re-usage (MR)					
MR2	3467.75±713.09c	6.86±0.08a	0.37±0.02b	2.42±0.03a	10.68±0.11a
MR3	3533.42±947.77b	6.19±0.15b	0.42±0.04a	2.30±0.04b	9.60±0.20b
MR4	3597.72±823.14a	6.97±0.37a	0.38±0.04b	2.33±0.13b	10.64±0.55a
MR	***	***	**	**	***
Interactions					
MS × AMT	*	**	***	**	***
MS × MR	*	***	***	***	***
AMT × MR	NS	**	***	***	***
MS × AMT× MR	*	***	***	**	***

Data: mean ± SE. Mean values in each column followed by at least one similar letter are not significantly different at α = 0.05 (Tukey's Test). **A1**: DFEC; **A2**: DFEC + IMF; **A3**: DFEC + HA; **A4**: DFEC + IMF + HA; **A5**: DFEC + NPK; **A6**: DFEC + NPK + IMF; **A7**: DFEC + NPK + HA; **A8**: DFEC + NPK + IMF + HA. *, **, or *** = Significant at α = 5%, 1%, or 0.1%. NS = Not-significant.

Changes in Micronutrient Content

Micronutrients of the DFEC before and after treatments are shown in Tables 2 and 5, respectively. MS×AMT×MR significantly affected the micronutrients (Na, Mn, Cu and Zn) of the DFEC (Table 5). MS×AMT, or MS×MR, significantly affected all analysed micronutrients. AMT×MR significantly affected the rest, but Fe. ST-DFEC had significantly higher micronutrients (Na, Mn, Fe, Cu and Zn) than NST-DFEC (Table 5). Of the control and amended DFEC, A1-A8 were not significantly different in Zn (Table 5). For Na, the trend was A8=A7=A2-A4≥A1=A6=A5. For Mn, the trend was A8>A2 and A8=A1=A3-A7 (Table 5). For Fe, it was A8=A7=A5>A1-A4=A6 (Table 5). The trend for Cu was A8=A4≥A5=A3≥A1=A2=A6=A7 (Table 5). The Na content across the

DFEC re-usage was not significantly different (Table 5). For Mn, Fe, and Zn, it was MR2=MR4>M3, and K was MR2=MR3>MR4. Generally, micronutrient availability decreased in a fluctuating pattern across the compost re-usage.

Table 5. Sterilization, HA and IMF amendments, and re-usage effects on DFEC's micronutrients.

Treatments	Na (ppm)	Mn (ppm)	Fe (ppm)	Cu (ppm)	Zn (ppm)
Media Sterilization (MS)					
NST	0.179±0.005b	0.184±0.005b	0.651±0.022b	0.008±0.000b	0.050±0.001b
ST	0.228±0.006a	0.240±0.006a	0.850±0.038a	0.011±0.000a	0.062±0.001a
NST vs. ST	***	***	***	***	***
Amendments (AMT)					
A1	0.196±0.010bc	0.209±0.012ab	0.672±0.051c	0.010±0.001bc	0.054±0.003a
A2	0.207±0.013abc	0.204±0.013b	0.588±0.050c	0.009±0.001c	0.055±0.004a
A3	0.198±0.010abc	0.212±0.012ab	0.633±0.069c	0.010±0.001abc	0.055±0.003a
A4	0.218±0.010ab	0.223±0.009ab	0.677±0.047c	0.010±0.000ab	0.058±0.002a
A5	0.189±0.011c	0.212±0.016ab	0.844±0.069ab	0.009±0.001bc	0.055±0.003a
A6	0.192±0.011bc	0.205±0.012ab	0.747±0.057bc	0.009±0.001c	0.055±0.003a
A7	0.206±0.014abc	0.207±0.013ab	0.892±0.065ab	0.009±0.001c	0.056±0.003a
A8	0.223±0.014a	0.224±0.013a	0.951±0.073a	0.011±0.001a	0.060±0.003a
AMT vs. AMT	**	*	***	***	NS
Media Re-usage (MR)					
MR2	0.202±0.007a	0.217±0.002a	0.734±0.025b	0.010±0.000a	0.058±0.001a
MR3	0.206±0.003a	0.199±0.005b	0.694±0.033b	0.010±0.000a	0.053±0.001b
MR4	0.203±0.007a	0.220±0.012a	0.823±0.056a	0.009±0.001b	0.056±0.003ab
MR vs. MR	NS	***	**	***	**
Interactions					
MS × AMT	*	**	***	***	*
MS × MR	***	***	***	***	***
AMT × MR	***	*	NS	***	***
MS × AMT× MR	**	*	NS	*	**

Data: mean ± SE. Mean values in each column followed by at least one similar letter are not significantly different at $\alpha = 0.05$ (Tukey's Test). **A1**: DFEC; **A2**: DFEC + IMF; **A3**: DFEC + HA; **A4**: DFEC + IMF + HA; **A5**: DFEC + NPK; **A6**: DFEC + NPK + IMF; **A7**: DFEC + NPK + HA; **A8**: DFEC + NPK + IMF + HA. *, **, or *** = Significant at α = 5%, 1%, or 0.1%. NS = Not-significant.

DISCUSSION

Effects of Sterilization by Autoclaving

Autoclaving improves the agronomic physicochemical properties of DFEC (Table 3). Autoclaving affects soil's or media's physicochemical and biological properties (Trevors, 1996; McNamara *et al*., 2003; Tanaka *et al*., 2003). The slight increase in pH after autoclaving can be attributed to ammonium accumulation in the compost. Ammonium accumulates in autoclaved media because the ammonium-oxidising bacteria lose, while the dead microorganisms decompose (Berns *et al*., 2008). The ideal pH for better crop growth and yield ranges from 5.5 to 6.5 (Ingram, 2014), meaning the pH after autoclaving is still within the ideal range. Autoclaving also induces the split of different bonds in humic materials, causes a release of various ions, and increases the EC (Razavi & Lakzian, 2007). Moreover, autoclaving increases Mn, ammonium, organic N, OM, and OC contents (McNamara *et al*., 2003; Berns *et al*., 2008) but can also cause the opposite. Autoclaving changes the compost's chemical structure, leading to higher or lower OM and OC

(Berns *et al*., 2008). A similar structural change may have happened to the autoclaved DFEC but resulted in a lower OM and OC.

Autoclaving accelerates the decomposition of OM, leading to a higher macro-micronutrient release, thus improving nutrient availability in crop media (Berns *et al*., 2008). So, it is expected that ST-DFEC has better macro-micronutrients than NST-DFEC, except for N (see Tables 4 and 5). The latter is unexpected, but it can be that the amount of N released due to autoclaving is too small to affect the total N in DFEC. Compost derived from dairy farms could naturally be rich in N due to the animal protein-rich diets, urinary excretion, microbial activity, and OM decomposition (Kebreab *et al*., 2002), as shown in Table 4, so a little N increment is insignificant. Autoclaving enhances the agronomic quality of DFEC faster, but it may also shorten the compost's longevity because it accelerates decomposition.

Effects of HA and IMF Amendments

It is expected that A1-A4 were significantly higher in pH but lower in EC than A5-A8 (Table 3) because A5-A8 was added with NPK 15:15:15. NPK fertiliser in soil could interact with ammonium-N that can undergo nitrification, that is, when existing bacteria convert ammonium to nitrate, which releases hydrogen (H^+), thus increasing acidity. Phares and Akaba (2022) reported that NPK-amended soil decreased significantly in pH due to the transformation of NPK in soil and the release of H^+. The higher EC in A5-A8 could be due to the salt solubility found in NPK fertiliser. NPK fertiliser typically contains soluble salts, such as ammonium nitrate, potassium chloride and superphosphate. When these fertilisers are added to compost, they dissolve in the moisture present, increasing the concentration of ions, such as ammonium (NH_4^+), nitrate (NO_3^-) and potassium (K^+). The presence of these ions enhances the overall ionic strength of the compost mixture, thus increasing its EC. Makhlof *et al*. (2022) reported a noticeable rise in EC level in compost added with NPK fertiliser due to the added soluble ions from the fertiliser. The ideal media's pH for better crop growth and yield is 5.5–6.5 (Ingram, 2014). For EC, the recommended value varies depending on the type of growing medium, but for leafy vegetables, such as Pak Choy grown in soil, it is recommended to keep the EC below 2 mS/cm (Sun *et al*., 2019). So, as expected, NPK addition (A5-A8) reduces pH and increases EC, or in other words, improves the agronomic physicochemical properties of DFEC.

HA or IMF amendment does not affect DFEC's C/N and WHC, as A1-A8 were not significantly different in these factors (Table 3). Also, it does not affect DFEC's OM and OC contents because A1, A2, A4, A5, A6 and A8 were not significantly different in OM and OC (Table 3). However, A3 (DFEC+HA) had markedly lower OM and OC, while A7 (DFEC+NPK+HA) had significantly higher OM and OC than the rest (Table 3). The scenario in A3 indicates that HA addition alone lowers DFEC's OM and OC contents. This is because HA is beneficial in stimulating microbial activity and subsequently accelerating the decomposition and release of essential nutrients for plant uptake, reducing OM and OC levels in the media (Liu *et al*., 2022). The increase in microbial activity can lead to a faster breakdown of compost's OM and a consequent reduction in OC (Liu *et al*., 2022). This process is more noticeable as the compost favours high microbial efficiency, which can break down OM rapidly (Ni *et al*., 2024). Higher levels of OM and OC in growing media are essential for nutrient storage (Gerke, 2022). Hence, the data in A3 indicate that HA addition alone could improve plant nutrient uptake but shorten the effective life span of the compost, as it accelerates decomposition and OM and OC reduction. In A7, the data indicate that NPK addition enhanced the impact of HA on some aspects of the DFEC physicochemical properties, such as OM and OC contents. Okonwu and Mensah (2012) reported a similar result. As NPK fertiliser breaks down, more essential nutrients for microbial activity are released. The increase in microbial

activity leads to the decomposition of OM and could contribute to the higher OM and OC in the DFEC. Even so, NPK addition alone (A5) did not change DFEC's physicochemical properties compared to the control (A1) (Table 3), and regarding physicochemical property improvement, NPK+HA+IMF addition (A8) is no better than NPK+HA (A7). That trend is expected because the effect of NPK addition is more on the macronutrients of the DFEC.

IMF addition showed a trend to suppress HA's effect on DFEC's physicochemical properties (OM, OC, WHC) when there was NPK addition; for example, A7 (DFEC+NPK+HA) was better than or equal to A8 (DFEC+NPK+HA+IMF) in the studied parameters (Table 3). The added IMF could produce enzymes like oxidases, peroxidases, and hydrolases that break down complex organic molecules, including humic acids, into simpler forms. This process accelerates the decomposition of humic substances, reducing their beneficial effects on soil structure (Lehmann & Kleber, 2015).

The effects of HA and IMF amendments on DFEC's macronutrients (Table 4) can be interpreted as follows. The HA and IMF amendments do not affect the total N content of DFEC, as A1-A8 were not significantly different in total N concentration. That also means the NPK addition has little impact on total N in the compost. NPK, NPK+HA, NPK+IMF or HA+IMF addition (A4-A7) enhances P availability, and the effect is even better when NPK+HA+IMF (A8). Even so, IMF or HA addition alone is ineffective in improving P release (A2 and A3) (Table 4). NPK addition (A5-A8) increases K in the compost rather than HA, IMF and HA+IMF additions, as A1-A4 was significantly lower in K than A5-A8. NPK addition decreases Mg in the compost even with HA or IMF application (A5-A8), as A1-A4 was markedly higher in Mg than A5-A8. NPK+HA+IMF (A8) and IMF+HA (A4) enhance Ca availability, but IMF without HA (A2 and A6) does not, which means HA is essential for higher Ca release in the compost. For DFEC's micronutrients (Table 5), the effects of HA and IMF amendments show the following interpretations. NPK addition enhances Fe availability (A5-A8); the effect is better with IMF or HA application. Only NPK+HA+IMF application (A8) enhances Na, Mn or Cu availability, demonstrating that NPK+HA+IMF is essential for improving the availability of these elements. However, HA and IMF amendments, even with NPK addition, do not affect Zn availability in the compost, as A1-A8 were not significantly different in Zn.

In the literature, the general trends are that HA and IMF amendments improve the media's macro-micronutrients. Hence, the present study's P, K, Ca, Fe, Mn and Cu increments are expected. P and K were directly increased in the DFEC through NPK fertiliser application. The DFEC naturally contains various beneficial microbes, such as P-, K- and silicate-solubilising bacteria (Basri *et al.*, 2021). These bacteria could emit P and K from P-bearing and K-bearing minerals in soils (Parmar & Sindhu, 2013). The ability of the silicate-solubilising bacteria to solubilise silica could facilitate the release of other nutrients, such as P, K, Fe, Ca, Mg and Na (Raturi *et al.*, 2021). Composts from dairy manure contain abundant essential macro- and micronutrients that can be solubilised into accessible form (Stowell & Bickert, 1995). HA is known to attract nutrients and allow better dissolvent of nutrients, which increases soil productivity (Li *et al.*, 2019b; Belal *et al.*, 2019). Meanwhile, microbial activities within IMF can facilitate the mobilisation of micronutrients, such as Fe, Zn, Cu, and Mn (Singh *et al.*, 2022). When combined with IMF, HA stimulates microbial activity, leading to increased mineralisation of OM and release of nutrients (Plaza *et al.*, 2005). The combined effect was even reflected in crop agronomic improvement. Radius (2024) reported that CDP planted in DFEC added with both HA and IMF was 37.86 g/plant compared to 35.97 g/plant in the control, and HA+IMF without NPK addition was sufficient to achieve an acceptable CDP yield per plant.

However, the P, K, Ca, Na, Fe, Mn and Cu increments were significant only with NPK fertiliser application, meaning HA, IMF or HA+IMF addition alone is ineffective in significantly improving the DFEC's agronomical quality. Holatko *et al*. (2022) reported that HA addition alone does not substantially affect maturated cattle manure's N, P, K, Mg and Ca levels. On the other hand, Dhaliwal *et al*. (2019) found that NPK 15:15:15+HA significantly increases macro-micronutrient release and availability in growing media. That explains the effect of NPK fertiliser application on the DFEC's macro-micronutrients, especially in A8, where OM and OC are also the highest. Higher OM and OC are essential in storing nutrients (Gerke, 2022). IMF and HA addition primarily improve the compost's macro-micronutrients by enhancing the bioavailability of nutrients in the DFEC. Even so, these two components do not directly increase the nutrients in DFEC. In contrast, NPK fertiliser directly supplies the nutrients in ready form. That explains the ineffectiveness of the HA, IMF or HA+IMF addition without NPK fertiliser. The latter does not deny the long-term effect of HA, IMF or HA+IMF in significantly increasing crop productivity.

It is also unexpected that N and Zn were insignificantly different in A1-A8, and NPK addition decreased Mg content. The N released due to the HA and IMF amendments is probably too little to affect the total N in the DFEC since the compost naturally has high N content (see total N in Table 2 and A1 in Table 4). A 100% compost derived from animal wastes contains a high N level (Sudita *et al*., 2021). Font-Palma (2019) reported that the N level in cattle manure was 22000 ppm, comparable to the amount reported in the present study before NPK addition (Table 2). Also, it can be that the Pak Choy planted on the compost immediately took up the released N, creating a constant N in A1 to A8. The insignificantly different Zn levels found across the different HA and IMF amendments could be due to Zn's chemical properties. It can form stable compounds with OM in compost (Al Chami *et al*., 2013). During the composting process, Zn's initial transformation and stabilisation might reach a stability point where additional HA, IMF and NPK applications no longer significantly affect the Zn level. NPK addition suppressed the Mg level (Table 4), as K and Mg are positively charged cations (K^+ and Mg^{2+}) and compete for uptake by plant roots. A high K level decreases Mg uptake (Xie *et al*., 2021). Hence, more Mg remains in the soil. However, being a cation, Mg is prone to leaching, leading to reduced soil Mg. The lower Mg content in A5 (without HA application) demonstrated that trend. In contrast to A5, more Mg was at least retained in A7 and A8 because the HA addition allowed better chelation of Mg ions and stimulated microbial activity. The IMF addition further enhanced the breakdown of OM and facilitated the release of cations, such as Mg, to replace the leached ions.

All analysed macro-micronutrients found across the HA-IMF amended DFEC were lower than the values in cattle manure compost reported by Anwar *et al*. (2017). This trend indicates that macro-micronutrients vary for different cattle manure-based composts. The difference might be due to several factors, such as the composition of the cattle manure originating from the cattle diet, the composting method or the handling and storage of the compost before being analysed.

Effects of Re-usage

The effects of compost re-usage on the DFEC's physicochemical properties and macro-micronutrients closely reflect the outcomes of continuous decomposition as the compost ages. The DFEC's physicochemical properties deteriorate at every re-usage (Table 3). Vegetable data indicated that even when mixed with soil, DFEC decomposed continuously and incrementally over time as it was reused (Maludin *et al*., 2019). Nitrification during DFEC's decomposition could have lowered the pH across MR2 to MR4. Nitrification increases at the compost's curing stage, releasing more hydrogen ions (H^+) and decreasing pH. The EC increment during the third re-usage of DFEC could be due to further OM decomposition and reduced compost volume. Decomposition

and decreased media volume concentrate the remaining salts and nutrients and increase EC (Insam & Bertoldi, 2007). OM decomposition also causes a decline in OM and OC upon each successive reuse of compost. At every reuse, the microbial activity in the compost enhances the decomposition of the remaining organic material, resulting in a continued decline in OM and OC (Bernal *et al*., 2009). Soluble organic compounds can also be lost through leaching during composting and subsequent uses, especially in conditions with high moisture. These losses contribute to compost's overall decline in OM and OC contents (Tiquia *et al*., 2002). Aggelides and Londra (2000) reported that compost WHC decreases significantly over time due to decomposition of OM and structural changes within the compost. Compost undergoes ongoing decomposition and humification processes, breaking the OM into finer particles and resulting in a loss of structure and porosity, initially contributing to high WHC. That could explain the reduction of DFEC's WHC across MR2 to MR4.

The OM, OC and WHC trends could explain the DFEC's macro-micronutrient trends concerning the compost re-usage. Soils with higher OM and WHC have better nutrient storage (Williams *et al*., 2016; Gerke, 2022). Hence, the lowest OM and OC in MR3 (Table 3) explains that MR3 has the lowest macro-micronutrients (Tables 4 and 5). The declining trend in WHC throughout the compost re-usage (Table 3) describes the reduction in macro-micronutrient availability from MR2 to MR4 (Tables 4 and 5). Maintaining high WHC is crucial for nutrient retention in soils and composts, making the nutrients available longer (Alkharabsheh *et al*., 2021).

N increased over time across MR2 to MR4 (Table 4). This increment corresponded with the reduction in DFEC's C/N ratio (Table 3). C/N ratio and N release have a strong relationship; a high C/N ratio promotes N immobilisation, and a low C/N ratio promotes N mineralisation (Chaves *et al*., 2005). A reduction in the C/N ratio over time means an increment in N mineralisation (Parnaudeau *et al*., 2006). N mineralisation is a decomposition of N into plant-accessible forms, such as NH_4^+ through ammonification and NO_3^- through nitrification. Al-Bataina *et al*. (2016) also reported that compost's N and P content (%) increases as compost ages. Hence, the C/N ratio, which affects the N release, is also expected to affect the release of the other macro-micronutrients over the re-usage of the DFEC.

CONCLUSION

Sterilization increased the pH, EC, and macro-micronutrients, except for N, and decreased the OM and OC of DFEC. This trend indicates that sterilization enhances decomposition (OM and OC decreased) and, thus, increases the EC and macro-micronutrients of the compost. The effects of HA and IMF amendments on DFEC's macro-micronutrients can be ranked as NPK+HA+IMF (A8) > NPK+HA (A7) > NPK+IMF (A6) > HA+IMF (A4) > HA (A3) > IMF (A2). The addition of either IMF (A2), HA (A3), or HA+IMF (A4) does not significantly improve the DFEC's physicochemical properties and macro-micronutrients. The vegetable data indicated that A4 is sufficient for an acceptable minimum yield, meaning HA+IMF improves plant nutrient uptake rather than the compost's physicochemical properties and macro-micronutrient content. The HA, IMF or HA+IMF addition is efficacious in improving DFEC's physicochemical properties and macro-micronutrients only when NPK is added (A5-A7), and the effect is even better when all are combined (A8). It is expected that some of the microbes in the compost are N-fixers and PK-solubilisers and able to expand their population when the fertiliser is added, leading to faster decomposition of the DFEC to release more nutrients in the compost. The effects of NPK addition alone fall within that of NPK+HA and NPK+IMF, which is expected because, to some extent, DFEC naturally contains HA and beneficial microbes. Re-using the DFEC led to the exhaustion of the compost's

physicochemical properties and macro-micronutrients over time. The latter trend aligns with the general understanding that compost continuously decomposes and releases more nutrients while deteriorating. The findings in the present study also reflect the situation in which the DFEC is under repeated HA, IMF or NPK applications, as the compost was re-applied with HA, IMF and NPK 15:15:15 at every vegetable cultivation cycle. It is also noted that the findings shall be interpreted cautiously, as many interaction effects in the data analysis are significant. The present study could be repeated to understand better the long-term effects of HA and IMF application on DFEC's physicochemical properties and macro-micronutrients and to determine the limit of DFEC re-usage.

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