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Synergistic effect of biochar amendment in milk processing industry sludge and cattle dung during the vermiremediation

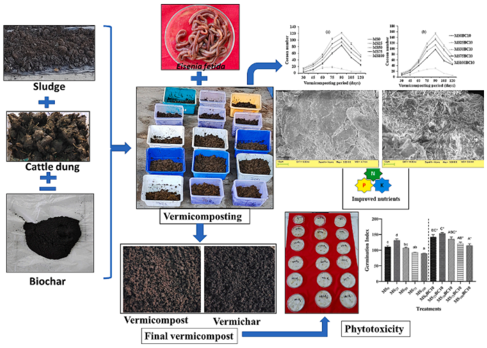
Rahil Dutta[a], Deachen Angmo[a], Jaswinder Singh[b,1], Anu Bala Chowdhary[a], Jahangeer Quadar[a], Sharanpreet Singh[a], Adarsh Pal Vig[a,1,*]

[a] *Department of Botanical and Environmental Sciences, Guru Nanak Dev University, Amritsar 143005, India*
[b] *P.G. Department of Zoology, Khalsa College Amritsar, Punjab, India*

HIGHLIGHTS

- Biochar acted as an effective additive for milk industry sludge waste vermicomposting.
- Addition of Biochar increased *Eisenia fetida* growth and reproduction.
- Biochar immobilized heavy metals during the vermicomposting period.
- Biochar aids in improvement of nutrients and enhanced mineralisation.

GRAPHICAL ABSTRACT



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ABSTRACT

The effective and sustainable management of fast growing and large quantities of industrial waste is a serious issue. The purpose of the present study was to assess the synergistic effect of biochar (BC) amended in milk processing industry sludge (MS) mixed with cattle dung (CD) in different ratios through vermiremediation. The MS_{25} and $MS_{25}BC_{10}$ (25:75 + 10 % BC) showed the least mortality and greatest earthworm growth and development. The final product from all feed mixtures recorded a decrease in pH, total organic carbon and C/N ratio. Other parameters *viz.*, electrical conductivity, total available phosphorus, total Kjeldahl nitrogen, total sodium, total potassium and ash content was observed to be increased after vermicomposting. Significantly lower heavy metal content was found in all biochar amended feed mixtures than in mixtures without biochar. The germination index of *Trigonella foenum-graecum* showed a value ranging from 89.14 to 131.46 % for mixtures without BC and 115.18–153.47 % for biochar amended mixtures.

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* Corresponding author.
E-mail addresses: rahildutta02@gmail.com (R. Dutta), singhjassi75@yahoo.co.in (J. Singh), dr.adarshpalvig@gmail.com (A. Pal Vig).

[1] These Authors shared equal contribution.

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1. Introduction

The unprecedented growth in the industrial sector has resulted in deterioration of every sphere of the environment *viz.,* water, soil and air (Karthika et al. 2022). The food and dairy industry, more than any other sector, has a significant contribution to the degradation of the environment due to generation of enormous amounts of waste during manufacturing and processing of food (Shi et al. 2021). The dairy industry sector is a key constituent of the food industry in India with an annual milk output of 192 million tonnes (OECD/FAO, 2020). According to the OECD-FAO Agricultural Outlook 2020–2029 report, the global milk production is expected to grow at 1.6 percent per year by 2029. Due to the presence of macro and micronutrients, dairy industry sludge is commonly used in agricultural fields (Ryan and Walsh, 2016). However, the possible environmental effects of dairy sector sludge have not been well studied, and as a result, these products cannot be recognized as being both profitable and sustainable (Shi et al. 2021). Attributed to the prevalence of heavy metals, toxic organic compounds, microbes, and other dangerous substances, direct application of sludge into the soil might be potentially detrimental (Liew et al. 2022). Biological, chemical, and physical contamination can occur at any point within the dairy supply chain. Hazardous contaminants such as microbes, fungi, heavy metals, pesticides, organic toxins, and veterinary drugs get concentrated in the sludge during the sewage treatment of the milk industry (Montgomery et al. 2020). The treatment and disposal of wastewater sludge and effluents from the milk processing sector pose a challenge for sustainable management. Existing disposal techniques for milk processing industry sludge (MS) waste such as landfilling and open dumping, are not only becoming more costly but also unfeasible as open space becomes scarce (Kaza et al. 2018). However, the environmental impacts can be reduced to a greater extent by the stabilization of sludge before dumping (Liew et al. 2022). As a result, treating dairy wastewaters and sustainably addressing increased sludge formation would be a greater challenge.

Vermicomposting has been accepted as an environmentally beneficial waste stabilization process for transforming organic wastes into high-value organic manure (Mago et al. 2021).Vermiremediation has been proven to be a potentially sustainable process for reducing the bioavailability and toxicity of heavy metals in waste (Mondal et al. 2020; Paul et al. 2020a; Hussain et al. 2021). Previous studies indicate that the use of various amendments to sludge prior to vermicomposting negates the effects of contaminants on the activity of earthworms. These amendments include phosphate rock (Wang et al. 2013), saw dust (He et al. 2016), fly ash (He et al. 2016), biochar (Malińska et al. 2017; Khan et al. 2019). The bioavailability of heavy metals is reduced by the amendment of biochar in course of sewage sludge vermicomposting that resulted in enhanced earthworm activity (Malińska et al. 2017) and increased concentration of macronutrients (Khan et al. 2019). The incorporation of biochar in the vermicomposting process has received very little attention (Khan et al. 2019). Biochar has gained international interest due to its broad usage in increasing soil productivity, soil quality, environmental remediation, and attaining carbon neutrality by lowering greenhouse gas emissions (Wang et al. 2021b). Biochar is a black colour material produced by the pyrolysis of organic biomass such as forestry, agricultural, municipal, green, and food waste (Chi et al. 2021). The organic carbon and water holding capacity of soil are improved by the addition of biochar (Wang et al. 2021a). Due to the presence of various macro and micronutrients, biochar can serve as an additional source of nutrients for soil microbes and macrofauna such as earthworms (Lehmann et al. 2011). Several studies reported that the addition of biochar led to the enhancement in organic matter degradation, physico-chemical properties and microbial activities. Furthermore, the biochar amendment reduces the nitrogen losses and emissions of various greenhouse gases during vermicomposting and composting of biological waste (Paul et al. 2020b). Some reports have concluded that biochar amendment to the substrate has a positive influence on the entire chained composting and vermicomposting process (Malińska et al. 2017; Khan et al. 2019). Amendment of biochar during biowaste vermicomposting aids in the development, proliferation, and persistenceof earthworms, resulting in the effective transformation of waste into a valuable resource and, eventually, boosting the characteristics of finished vermicompost (Malińska et al. 2017).

So far, no extensive information is available regarding the vermicomposting of milk processing industrial sludge by using cattle dung and paddy straw biochar (BC) as an amendment. Considering all these aforementioned factors into account, the following objectives have been selected for the current investigation (a) to study the effects of milk processing industry sludge in different ratios with cattle dung and biochar on earthworm development and reproduction during vermicomposting; (b) to analyse the physicochemical properties to assess nutrient content and to ascertain the heavy metal content in the varied feed combinations (initial and final); (c) determine the phytotoxicity and maturity of vermicompost using seed germination index, SEM and FT-IR analysis.

2. Materials and methods

2.1 Collection of milk industry sludge

The dewatered milk processing industry sludge (MS) was collected from Verka milk industry, Amritsar (Punjab), India. The sludge was shade dried-up for 14 days to remove the excessive moisture and foul smell. It was crushed to uniform size so that worms may devour it more easily, either on its own or when combined with cattle dung (CD).

2.2 Cattle dung and earthworm collection

Fresh cattle dung free from the urine was procured from the local dairy. The partially dried cattle dung was homogenized well for 14 days so that any toxic gases present may escape and to remove the excess heat that can affect the survival of earthworms.

Young hatchlings (without clitellum) of equal size and weight were arbitrarily hand-picked from the stock culture of *Eisenia fetida* fed with decomposed cattle dung, maintained at the vermicomposting unit situated at Botanical garden, Guru Nanak Dev University, Amritsar (Punjab), India.

2.3 Preparation of biochar

The biochar (BC) was prepared by the pyrolysis of paddy straw. The straw was collected from the Guru Nanak Dev University agricultural fields and air dried for 10 days to reduce the moisture. The straw was then chopped into small pieces and pyrolyzed in self designed double chambered kiln at 300 to 450 °C for 1 h. Following pyrolysis, the BC was crushed to the size less than 2 mm using grinding machine and stored for further use.

2.4 Experimental setup

For the first phase of experiment, different proportions of sludge were made by blending it with CD, MS:CD i.e. 0:100 (MS_0), 25:75 (MS_{25}), 50:50 (MS_{50}), 75:25 (MS_{75}) and 100:0 (MS_{100}). For the second phase of the experiment, the 10 % biochar was selected for efficient vermicomposting as described by Khan et al. (2019).The above mentioned ratios of MS:CD were amended with 10 % biochar (BC_{10}) i.e MS_0BC_{10}, $MS_{25}BC_{10}$, $MS_{50}BC_{10}$, $MS_{75}BC_{10}$, $MS_{100}BC_{10}$ (Table 1). Rectangular plastic trays of dimensions (26 × 23 × 8 cm) in triplicate at vermicomposting unit of the university were used for loading of the above waste mixtures (1Kg/tray). The trays were covered with jute bag, turned manually after every 24 h for 15 days for eradication of noxious gases, thermal stabilization and initial microbial degradation. After 15 days of precomposting, 20 young *Eisenia fetida* earthworms without

Table 1
Various proportions of milk processing industry sludge with cattle dung and biochar.

Feed mixtures	Cattle dung (kg)	Milk industry sludge (kg)	Biochar (%)
Control (MS_0)	1.00	0	0
MS_{25}	0.75	0.25	0
MS_{50}	0.50	0.50	0
MS_{75}	0.25	0.75	0
MS_{100}	0	1.00	0
Control (MS_0BC_{10})	1.00	0	10
MS_{25} BC_{10}	0.75	0.25	10
MS_{50} BC_{10}	0.50	0.50	10
MS_{75} BC_{10}	0.25	0.75	10
MS_{100} BC_{10}	0	1.00	10

clitellum were introduced in each tray. Throughout the experiment, a temperature range of 24–37 °C was noted and the moisture content was kept at 60–70 % by regular sprinkling the area with adequate water. During the experimental period of 120 days, the earthworms, their cocoons and hatchlings were sorted manually at an interval of every 15 days and the number of earthworms, cocoon and hatchlings was noted along with the earthworm biomass. After 120 days (termination day) the earthworms, cocoons and their new generations were separated. The final vermicompost samples were sorted, sieved, dried in the air and packed in the air tight bags for physico-chemical parameters, heavy metal analysis, phytotoxicity, stability and maturity of vermicompost. The initial physicochemical properties of cattle dung, milk industry sludge and biochar were also evaluated (See Supplementary material).

2.5 Physico-chemical analysis

All initial and final collected samples were subjected to physico-chemical analysis to assess the stability and maturity. The pH and electrical conductivity (EC) were calculated using extracts of each sample in distilled water (DW) at a ratio of 1:10 (w/v) utilizing Systronics pH/EC/TDS-372 meter. The method of Total organic carbon (TOC) was adopted from Nelson and Sommers (1996) and Quadar et al. (2022) with minor modifications. Total Kjeldahl Nitrogen (TKN) was measured by micro-Kjeldahl method (Bremner and Mulvaney, 1982) following digestion of samples with digestion mixture (H_2SO_4 and K_2SO_4, $CuSO_4$, SeO_2 in 10:4:1). Total available phosphorus (TAP) was measured by synergy H1 microplate reader following digestion of samples in di-acid mixture ($HClO_4$/HNO_3 in ratio 1/4). Total potassium (TK) and Total Sodium (TNa) were quantified by systronics flame photometer. The Carbon:Nitrogen (C/N) ratio was calculated by dividing C and N content of the sample. The heavy metals (HM) *viz.,* Chromium (Cr), Copper (Cu), Cadmium (Cd), Manganese (Mn), Lead (Pb) and Zinc (Zn) were analysed following di-acid digestion method ($HClO_4$: HNO_3 in ratio 1:4) in ICP-OES.

2.6 Structural and stability characterisation

2.6.1 Scanning electron microscopy (SEM)

The texture of the initial feed mixtures and post vermicompost samples was examined by Carl Zeiss Supra 55 SEM in accordance to approach outlined by Bhat et al. (2014).

2.6.2 Fourier Transform-Infrared spectroscopy (FT-IR) analysis

The FT-IR spectroscopic technique was used to confirm the degradation, reduction or increase in phenolic compounds, aliphatic compounds, carboxylic groups, polypeptides and polysaccharides during the whole process of vermicomposting. The FT-IR spectra of vermicompost samples were recorded using the spectral range (400–4000 cm^{-1}) following the method of Quadar et al. (2022).

2.6.3 Phytotoxicity assay

The germination index (GI) of *Trigonella foenum-graecum* seeds was assessed to evaluate the maturity, stability and phytotoxicity of the final vermicompost. For this, 1 g of air-dried sample from each tray was collected and dissolved with 10 mL of distilled water. The obtained extracts were then stirred in shaker for 40 min and left for 72 h which was then filtered, thus obtaining supernatant. The germination test was conducted in Petri dishes with filter papers by placing 10 seeds in them. Then, 5 mL of vermicompost filtrate was added on each Petri dish and in the control variant only distilled water was added. The test was run in triplicate. All dishes were kept in dark at 24 ± 1 °C for five days of exposure. The germination index (GI) was calculated following Ahmed and Deka (2022).

2.7 Statistical analysis

The whole experiment was investigated in triplicate ($n = 3$) and the data is represented as Mean ± Standard Error (S.E.). The significant changes in the growth of worms and the study of phytotoxicity of varied feed treatments were driven by one-way ANOVA followed by Tukey's test. Initial and final samples of vermicompost were compared by Student's *t*-test for physicochemical and heavy metals. Independent *t*-test was used to compare the various parameters of different feed mixtures with and without biochar. The computer software tool SPSS (version 16.0) was used to conduct the statistical analysis.

3 Results and Discussion.

3.1 Effect of sludge on survival, growth, reproduction and fitness of earthworm

In the present study, the vermicomposting of sludge was done in various mixtures with cattle dung and biochar. The different feed mixtures resulted in considerable variations in various developmental parameters of *E. fetida.*

3.1.1 Earthworm number and biomass

The earthworm population growth varied significantly with both treatments ($p \leq 0.05$) and experimental days ($p \leq 0.05$). On 60th day, the earthworm's number began to increase in $MS_{0,}$ MS_{25}, MS_{50} and MS_{75} and it extended till the 90th day of the experimental period with the maximum increase in MS_{25} (35.00 ± 1.00) and minimum in MS_{75} (27.00 ± 0.58) (Fig. 1a). However, in MS_{100} mixture, the earthworm number decreased right from the 15th day of the experiment with a value of 15.67 ± 0.67 earthworms on 90th day. The worm number on 90thday follow the order of $MS_{25} > MS_0 > MS_{50} > MS_{75} > MS_{100}$. The number of earthworms declined after 90th day in all the treatments due to nutrient exhaustion for earthworms in the feed mixtures (Gusain and Suthar 2020a).

Further, the earthworm biomass also exhibited significant variations with both treatments ($p \leq 0.05$) and experimental days ($p \leq 0.05$). The highest earthworm biomass was found in the MS_{25} (29.53 ± 0.74 g) on 90th day of the experiment, while lowest was found in the MS_{100} (5.47 ± 0.35 g) on 90th day of the experimental period (Fig. 1c). The present results comply with Suthar (2012) who reported that 40–60 % of milk industry wastewater sludge can be converted into vermicompost without any adverse effects on earthworms.

The increase in the number and biomass of earthworms observed in the present study is in accordance to Quadar et al. (2022) who reported a similar increase in the number and biomass of earthworms during vermicomposting of coconut husk blended with cattle dung. The resultant increase in earthworm number and biomass upto 90th day in all treatments except MS_{100} could be accredited to ideal feed or availability of sufficient nutrients in feed mixtures upto 90th day. Negi and Suthar (2018) also reported that the kind, palatability and food quality had a

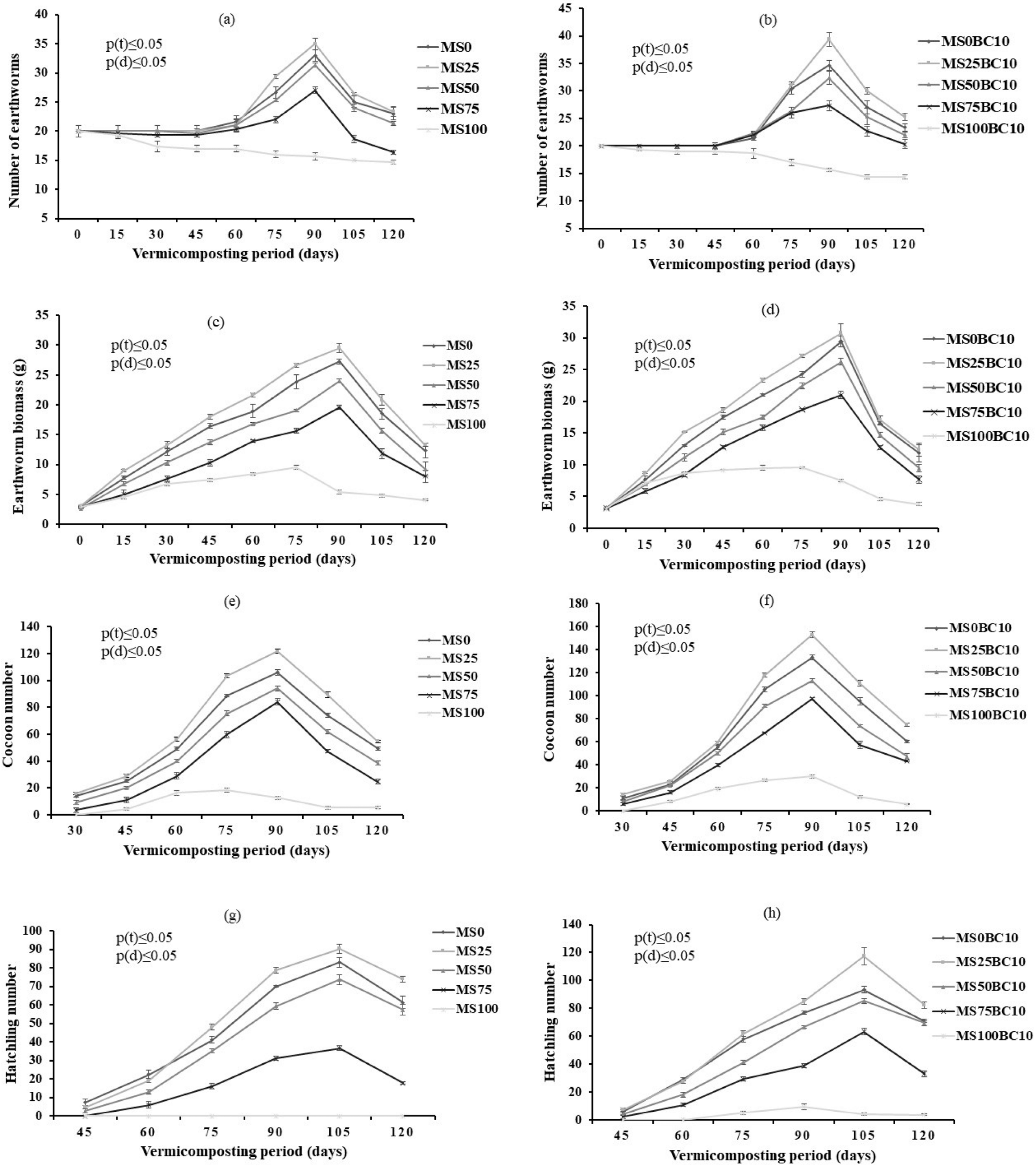

Fig. 1. Mean values of number of earthworm (a) without and (b) with biochar; earthworm biomass (c) without and (d) with biochar; cocoon number (e) without and (f) with biochar; hatchling number (g) without and (h) with biochar in different feed mixtures during vermicomposting periods.

direct effect on earthworm reproduction, development, and survival. The decline in the biomass and earthworm number after 90th day might be due to the increased earthworm density, ageing of raw materials, reduction of bioavailable nutrients, food exhaustion and the transformation of majority of the used substrate to vermicompost (Li et al. 2020).

3.1.2 Cocoon number and hatchling formation

The cocoon number was also found to be significantly ($p \leq 0.05$) influenced with varied feed treatments and different time intervals. The cocoon began to appear on 30th day in MS_0, MS_{25}, MS_{50} and MS_{75} and afterwards 45th day in MS_{100}. It was observed that the number of cocoons increased with time and progressed upto 90th day in all the treatments except with MS_{100} where the cocoon number increased only upto 75th day. On 90th day of the experiment, the highest cocoon number was noticed in MS_{25} (121.67 ± 1.76) and the minimum cocoon numbers were observed MS_{100} (12.67 ± 1.20) (Fig. 1e). The cocoon production was in the order of $MS_{25} > MS_0 > MS_{50} > MS_{75} > MS_{100}$ on 90th day. Thereafter, the cocoon production decreased in all the treatments which may be due to exhaustion of food with time. Cocoon production is also directly correlated to the growth of earthworms which, in turn, depends upon the biochemical characteristics of the feed mixtures (Gusain and Suthar, 2020b) with favourable feed promoting growth and development while unfavourable feed resulting in mortality, declined activity and fecundity, thereby, adversely affecting the entire process of vermicomposting. Our results are corroborated with findings of Bhat et al. (2014) who reported that the concentrations of pressmud higher than 25 % led to decreased number of cocoons and Gupta and Garg (2008) who suggested that 30–40 % of sludge concentration is ideal for vermicomposting.

Hatchling formation varied significantly with treatments ($p \leq 0.05$) and exposure period ($p \leq 0.05$). The hatchlings formation was noticed from 45th day in MS_0, MS_{25} and MS_{50} and on the day 60th in MS_{75} and it increased till 105th day of experiment except in MS_{100} where no hatchling formation was observed throughout the experimental period. On 105th day, the number of hatchlings appeared in different feed treatments followed in the order of $MS_{25} > MS_0 > MS_{50} > MS_{75} > MS_{100}$ with the highest quantity noticed in MS_{25} (90.33 ± 2.33) (Fig. 1g).The lowered production of hatchlings with feed mixtures MS_{50}, MS_{75} and MS_{100} having higher amount of sludge than MS_{25} could be correlated to the decline in cocoon formations at higher concentrations. The present findings are in parallel to the outcomes of Singh et al. (2014) who reported that increasing concentration of sludge significantly lowered the hatchlings production during vermicomposting process.

3.2 Comparative analysis of the effect of biochar (BC) amended feed mixtures and non-amended feed mixtures on growth, reproduction, survival and fitness of earthworm

Different combinations of sludge with cattle dung and biochar (BC) resulted in substantially different earthworm population growth with both treatments ($p \leq 0.05$) and experimental days ($p \leq 0.05$). On 90th day, the biochar amended feed mixture $MS_{25}BC_{10}$ was observed to have the maximum earthworm number (39.33 ± 1.20) and biomass (30.77 ± 1.41 g) while the feed mixture $MS_{100}BC_{10}$ had the lowest values for the same (15.67 ± 0.33 and 7.50 ± 0.15 g, respectively) (Fig. 1b, d). In comparison to treatments without BC, the biochar amended mixtures showed slightly higher number and biomass of earthworms but the differences were statistically non-significant ($p \geq 0.05$). The present findings are in parallel to Paul et al. (2020b) and Gong et al. (2018) who concluded that amendments of biochar during the vermicomposting elevated the number and biomass of earthworms in vegetable waste and green waste.

The cocoon number as well as number of hatchlings in the different feed mixtures with biochar were also found to be significantly different ($p \leq 0.05$) with both treatments and days. On the 90th day, the highest cocoon number were noticed in $MS_{25}BC_{10}$ (153.00 ± 2.31) and the least cocoon number was observed in $MS_{100}BC_{10}$ (30.00 ± 1.53) (Fig. 1f). The mixtures amended with biochar showed significantly higher number of cocoons for all treatments in comparison to feed mixtures without biochar ($p \leq 0.01$). The biochar amended $MS_{25}BC_{10}$ feed mixture showed 25.75 % higher cocoon number than non biochar MS_{25} feed mixture. Study conducted by Malińska et al. (2016) demonstrated that addition of 4 % and 6 % of biochar during vermicomposting resulted in 13 % and 66 % increased number of cocoons, respectively. Malińska et al. (2017) also indicated that the amendment of the biochar to sludge from sewage prior to composting raised the number of cocoons by 213 %. Similarly, Khan et al. (2019) stated that addition of biochar significantly increased the number of cocoons.

The hatchlings formation was recorded to be maximum at 105th day with the BC amended feed mixture in $MS_{25}BC_{10}$ (117.33 ± 6.01) (Fig. 1h). On the same day, the number of hatchlings recorded with $MS_{100}BC_{10}$ was 4.33 ± 0.88. For all treatments, the mixtures amended with BC showed significantly higher hatchlings number than mixtures without BC ($p \leq 0.05$). The $MS_{25}BC_{10}$ feed treatment showed 29.89 % higher hatchlings than non biochar MS_{25} feed mixture. Malińska et al. (2016) found that treatment having 4 % and 8 % of biochar led to higher number of hatchlings than in treatment with no biochar.

The increase in number and biomass of earthworms, cocoon production and hatchlings formation with the BC amended mixtures could be the result of the enhanced biochemical quality of feed substrates including increased water holding capacity, improved porosity and nutrient supply, immobilization of heavy metals and increased microbial activity which facilitated the growth, reproduction and the survival fitness of earthworms (Khan et al. 2019; Paul et al. 2020b).

3.3 Dynamics in physiochemical parameters and nutrients without biochar

pH is one of the vital parameters for compost operation as it impacts the microbial activity directly and their subsequent involvement in the feedstock mineralisation (Gusain and Suthar 2020b). During the present study, a significant decrease ($p \leq 0.05$) in pH was observed in final vermicompost samples in comparison to initial feed mixtures (Table 2). The rate of percent decrease was observed in the order of $MS_0 > MS_{50} > MS_{25} > MS_{100} > MS_{75}$. The decrease in pH for final vermicompost has previously been reported by Ahmed and Deka (2022). The reduction in pH may be due to the release of organic acids, CO_2, orthophosphates and nitrates during the process of vermicomposting (Ahmed and Deka 2022).

The final vermicompost product resulted from all the feed mixtures showed a significant increase (13.39–49.07 %) in levels of EC ($p \leq 0.05$) except for MS_{100} feed mixture where the increase was non-significant as compared to initial feed mixture. The maximum increase was observed in MS_{50} followed by MS_0, MS_{25}, MS_{75} and MS_{100} (Table 2). Except for MS_0, EC in vermicompost samples was below the threshold for plant toxicity (>4 mS cm^{-1}) as proposed by Gusain and Suthar (2020a). The increased EC content has previously been reported by many studies and probably caused by multiple factors including nitrification, acidification and accelerated mineralization of the organic matter occurring during the vermicomposting process, which increases the content of soluble salts (Gusain and Suthar 2020b; Wang et al. 2021b; Quadar et al. 2022).

Significant decrease ($p \leq 0.05$) in TOC was observed in final vermicompost samples from all the feed combinations except for MS_{100} where the decrease was non-significant. The overall decline in TOC was 38.02 % in MS_{25}; 32.38 % in MS_0; 31.50 % in MS_{50}; 24.40 % in MS_{75} and 13.04 % in MS_{100} (Table 2). The decline in TOC may be attributed to the processes like microbial respiration and degradation of organic carbon by earthworms discharging CO_2 during the vermicomposting process (Cai et al. 2022; Singh et al. 2022).

Ash content is a key factor in organic matter decomposition and mineralisation. A significant increase ($p \leq 0.05$) in ash content was observed in the final vermicompost samples of all the feed mixtures except for MS_{100} where the increase was non-significant. The overall increase in ash was 105.34 % in MS_0; 78.64 % in MS_{25}; 54.63 % in MS_{50}; 35.87 % in MS_{75} and 14.71 % in MS_{100} (Table 2). The increase in ash level shows high feedstock decomposition and mineralization which could be the sign of vermicompost maturity (Mago et al. 2022). Increment in ash content in the present study is supported by observations made by various previous studies (Malińska et al. 2016; Ahmed and Deka 2022).

At the end of the experiment the TKN increased significantly (2.17–2.92 %) in the final vermicompost samples ($p \leq 0.05$). The highest increase in TKN was observed in MS_0 (50 %) followed by MS_{25} (46.16 %) > MS_{50} (42.17 %) > MS_{75} (34.02 %) > MS_{100} (19.05 %) (Table 2). The increase in TKN as reported in the present study was in accordance with Negi and Suthar (2018). This rise in TKN during vermicomposting may have been caused by the nitrogenous excretory products, mineralization of organic matter, and relative loss of carbon (Deepthi et al. 2021; Quadar et al. 2022).

A key element defining the vermicompost's maturity is the C/N ratio. A C/N ratio less than 20 as in the present study (ranging 9.19–13.88) indicates compost maturity which could be utilized as an organic soil

Table 2
Nutrient changes (Mean ± S.E., n = 3) and percent change over initial (parentheses) in different mixtures of milk sludge and cattle dung with and without biochar.

Physico-chemical parameters		MS$_0$	MS$_0$BC$_{10}$	MS$_{25}$	MS$_{25}$ BC$_{10}$	MS$_{50}$	MS$_{50}$ BC$_{10}$	MS$_{75}$	MS$_{75}$ BC$_{10}$	MS$_{100}$	MS$_{100}$ BC$_{10}$
pH	Initial	8.15 ± 0.08	8.23 ± 0.03	8.06 ± 0.01	8.36 ± 0.05	7.89 ± 0.06	8.10 ± 0.02	7.80 ± 0.06	8.05 ± 0.06	7.49 ± 0.07	7.71 ± 0.04
	Final	7.28 ± 0.13** (-10.60)	7.42 ± 0.07** (-9.92)	7.42 ± 0.06** (-8.02)	7.35 ± 0.05** (-12.12)	7.11 ± 0.14* (-9.88)	7.16 ± 0.02** (-11.56)	7.24 ± 0.09* (-7.18)	6.97 ± 0.03** (-13.49)	6.93 ± 0.04* (-7.43)	7.13 ± 0.07* (-7.52)
EC (mS/cm)	Initial	3.22 ± 0.09	2.92 ± 0.03	2.92 ± 0.07	2.65 ± 0.06	2.51 ± 0.05	2.23 ± 0.05	2.21 ± 0.03	2.29 ± 0.03	2.12 ± 0.03	1.99 ± 0.07
	Final	4.36 ± 0.03** (35.16)	3.77 ± 0.06** (28.96)	3.85 ± 0.12* (31.96)	3.63 ± 0.04** (37.11)	3.74 ± 0.13** (49.07)	3.17 ± 0.02** (42.15)	2.61 ± 0.08* (18.28)	2.84 ± 0.06** (24.05)	2.40 ± 0.05 (13.39)	2.23 ± 0.03* (12.06)
TKN (%)	Initial	1.45 ± 0.06	1.24 ± 0.06	1.80 ± 0.02	1.40 ± 0.08	1.94 ± 0.06	1.66 ± 0.12	2.26 ± 0.06	2.08 ± 0.05	2.45 ± 0.07	2.29 ± 0.08
	Final	2.17 ± 0.11** (50.00)	2.05 ± 0.05** (66.04)	2.59 ± 0.04** (46.16)	2.43 ± 0.08** (73.33)	2.75 ± 0.09* (42.17)	2.57 ± 0.05* (54.93)	3.03 ± 0.09* (34.02)	2.94 ± 0.04** (41.57)	2.92 ± 0.13* (19.05)	3.38 ± 0.12* (47.96)
TOC (%)	Initial	44.37 ± 1.24	46.09 ± 0.46	39.10 ± 0.17	41.12 ± 0.50	36.79 ± 0.56	37.35 ± 0.42	34.52 ± 0.67	35.14 ± 0.15	30.74 ± 0.13	31.13 ± 0.37
	Final	30.00 ± 0.57* (–32.38)	31.23 ± 0.23** (–32.23)	24.23 ± 0.69** (-38.02)	24.70 ± 0.25** (-39.94)	25.20 ± 0.80* (-31.50)	25.83 ± 0.91** (-30.84)	26.10 ± 0.36** (-24.40)	26.77 ± 0.32** (–23.84)	26.73 ± 0.93 (-13.04)	26.67 ± 0.63** (-14.33)
C/N ratio	Initial	30.76 ± 1.31	37.50 ± 2.31	21.77 ± 0.37	29.60 ± 1.93	19.04 ± 0.68	22.81 ± 1.79	15.28 ± 0.55	16.94 ± 0.32	12.57 ± 0.42	13.64 ± 0.39
	Final	13.88 ± 0.65** (-54.86)	15.23 ± 0.45** (-59.38)	9.35 ± 0.12** (-57.04)	10.20 ± 0.27** (-65.55)	9.18 ± 0.50* (-51.77)	10.08 ± 0.49* (-55.81)	8.61 ± 0.15* (-43.62)	9.11 ± 0.11** (-46.24)	9.19 ± 0.33** (-26.94)	7.92 ± 0.47* (-41.97)
Ash (%)	Initial	23.51 ± 2.14	20.54 ± 0.79	32.59 ± 0.30	29.10 ± 0.86	36.57 ± 0.96	35.60 ± 0.73	40.48 ± 1.16	39.41 ± 0.27	47.00 ± 0.23	46.34 ± 0.64
	Final	48.28 ± 0.98* (105.34)	46.15 ± 0.40** (124.69)	58.22 ± 1.20** (78.64)	57.42 ± 0.43** (97.29)	56.56 ± 1.38* (54.63)	55.46 ± 1.56** (55.78)	55.00 ± 0.62* (35.87)	53.85 ± 0.55** (36.64)	53.91 ± 1.61 (14.71)	54.03 ± 1.09** (16.59)
TAP (%)	Initial	0.97 ± 0.07	1.09 ± 0.11	1.49 ± 0.14	1.33 ± 0.10	1.86 ± 0.11	1.45 ± 0.16	2.53 ± 0.14	2.36 ± 0.13	2.94 ± 0.09	2.60 ± 0.10
	Final	1.50 ± 0.08** (54.48)	1.90 ± 0.15* (73.55)	2.36 ± 0.12** (58.69)	2.34 ± 0.09* (75.74)	2.37 ± 0.11* (27.55)	2.44 ± 0.07* (68.32)	3.02 ± 0.07 (19.61)	3.09 ± 0.06 (31.18)	3.28 ± 0.10 (11.63)	3.33 ± 0.12 (28.31)
TK (%)	Initial	2.60 ± 0.09	2.65 ± 0.05	1.46 ± 0.13	1.64 ± 0.04	1.05 ± 0.12	1.15 ± 0.03	0.66 ± 0.06	0.71 ± 0.03	0.34 ± 0.01	0.58 ± 0.05
	Final	3.38 ± 0.18* (29.71)	3.44 ± 0.09** (29.69)	2.03 ± 0.05* (39.36)	2.25 ± 0.07** (37.20)	1.27 ± 0.16* (21.34)	1.63 ± 0.33 (42.44)	0.83 ± 0.06* (26.90)	1.07 ± 0.06* (50.45)	0.42 ± 0.01 (23.30)	0.86 ± 0.02* (48.26)
TNa (%)	Initial	1.49 ± 0.03	1.38 ± 0.05	1.14 ± 0.05	1.13 ± 0.03	1.04 ± 0.04	0.96 ± 0.03	0.84 ± 0.05	0.72 ± 0.03	0.76 ± 0.03	0.67 ± 0.04
	Final	2.97 ± 0.06** (98.88)	1.96 ± 0.05** (42.03)	2.51 ± 0.13* (119.53)	1.74 ± 0.11* (53.69)	1.73 ± 0.10* (67.20)	1.34 ± 0.06* (39.24)	1.52 ± 0.05** (81.67)	1.09 ± 0.05* (51.85)	1.37 ± 0.05* (79.48)	1.09 ± 0.01** (50.50)

Student's *t*-test was used to determine the degree of significance (*$p \leq 0.05$), (**$p \leq 0.01$).

addition (Gusain and Suthar 2020b). Considerable decline ($p \leq 0.05$) in C/N ratio was noticed in final samples of the vermicompost. C/N ratio in the various feed mixtures declined in the following order: MS$_{25}$ (57.04 %) > MS$_0$ (54.86 %) > MS$_{50}$ (51.77 %) > MS$_{75}$ (43.62 %) > MS$_{100}$ (26.94 %) (Table 2). A similar reduction of C/N ratio after the vermicomposting process has previously been reported by Biruntha et al. (2020). The process of decomposition and mineralization of organic carbon and nitrogen present in the original feedstock via the action of earthworms and microbial diversity causes the production of CO_2 and accessible nitrogen thereby decreasing the amount of carbon and increasing the amount of nitrogen. This in turn causes a decrease in overall C/N ratio as in the present study (Deepthi et al. 2021; Quadar et al. 2022).

The content of TAP improved in all vermicompost samples and it was significantly increased ($p \leq 0.05$) in all the feed mixtures except for MS$_{75}$ and MS$_{100}$ where the increase was non-significant. The highest increase in TAP was noticed in MS$_{25}$ (58.69 %) followed by MS$_0$ (54.48 %), MS$_{50}$ (27.55 %), MS$_{75}$ (19.61 %) and MS$_{100}$ (11.63 %) (Table 2). The present results are in line with earlier reports on the increment in TAP content in vermicomposting process (Deepthi et al. 2021). The current increase in TAP during the vermicomposting could be attributed to i) earthworms gut phosphate solubilizing bacteria, ii) the activity of enzymes (phosphatase and phytase) and mineralisation of organic matter by the coaction of microbes and earthworms (Deepthi et al. 2021; Singh et al. 2022).

The content of TK is an essential aspect in defining vermicompost's fertilising ability, and it is reported that earthworms boost nutrient content by improving potassium transformation to forms that plants can absorb (Singh et al. 2022). A significant increase in TK (0.42–3.38 %) content was observed in all feed mixtures ($p \leq 0.05$) except for MS_{100} that showed a non-significant increase. The percent increase in TK content was in the order of MS_{25} (39.36 %) > MS_0 (29.71 %) > MS_{75} (26.90 %) > MS_{100} (23.30 %) > MS_{50} (21.34 %) (Table 2). The present increase in TK was supported by Mago et al. (2021). According to Badhwar and Singh (2022), organic acid generation during the vermicomposting process causes K^+ ion dissolution in the mixtures which might have raised the TK concentration in treatment units.

Sodium is a crucial element that plays an important role in the regulation and maintenance of soil structure. In all the final vermicompost samples, significant increase ($p \leq 0.05$) in TNa content was observed. The percent increase in TNa content was in the order of MS_{25} (119.53 %) > MS_0 (98.88 %) > MS_{75} (81.67 %) > MS_{100} (79.48 %) > MS_{50} (67.20 %) (Table 2). The rise in TNa could be attributed to decreased feed volume and increased mineralisation of organic matter as a result of enhanced microbial and enzymatic activity by earthworms (Soobhany et al. 2015). Similar to the present study, increased levels of TNa content have been reported by Singh et al. (2022).

3.4 Synergistic effect and comparative analysis of physico-chemical and nutrient dynamics with and without biochar

A significant decrease ($p \leq 0.05$) in the pH of vermicompost samples from biochar amended mixtures was noticed following the order: $MS_{75}BC_{10}$ (13.49 %) > $MS_{25}BC_{10}$ (12.12 %) > $MS_{50}BC_{10}$ (11.56 %) > MS_0BC_{10} (9.92 %) > $MS_{100}BC_{10}$ (7.52 %) (Table 2). Wang et al. (2021b) reported a similar kind of reduction in pH after the composting of distilled waste with biochar. The decline in the value of pH can be ascribed to the formation of phenolic and organic compounds in the final stage of vermicomposting (Gong et al. 2018). No significant difference was observed in pH of final vermicompost in treatments with and without biochar amendment.

The EC values of final vermicompost were found to be significantly higher than the initial mixtures ($p \leq 0.05$). The percent enhancement of EC was in the order: $MS_{50}BC_{10}$ (42.15 %) > $MS_{25}BC_{10}$ (37.11 %) > MS_0BC_{10} (28.96 %) > $MS_{75}BC_{10}$ (24.05 %) > $MS_{100}BC_{10}$ (12.06 %) (Table 2). The results of the present study are consistence with Jain et al. (2018) who reported the increased EC values during the composting of BC amended mixture of *Hydrilla verticillata*, cow dung and sawdust. The decomposition of organic matter and the liberation of various salts in useful forms, such as potassium, ammonium, phosphate etc. may have contributed to this rise in EC (Gupta and Garg 2008). In comparison to vermicompost products without biochar, the EC values were slightly lower in biochar amended vermicompost samples in most of the treatment groups (Table 2).

The final product of all the feed mixtures showed a substantial reduction in TOC ($p \leq 0.01$). The percent reduction in TOC was in the series of 39.94 % in $MS_{25}BC_{10}$, 32.23 % in MS_0BC_{10}, 30.84 % in $MS_{50}BC_{10}$, 23.84 % in $MS_{75}BC_{10}$ and 14.33 % in $MS_{100}BC_{10}$ (Table 2). Comparatively, the maximum decline in TOC was noticed in vermicompost with biochar amendments than without biochar. For instance, $MS_{25}BC_{10}$ exhibited 39.94 % in TOC reduction while MS_{25} exhibited 38.02 % decrease in TOC from initial feed mixtures. The higher decrease in biochar amended mixtures could be due to increased earthworm and microbial activity. Consistent similar results of a decrease in TOC were reported by various researchers (Khan et al. 2019; Paul et al. 2020b; Cai et al. 2022). The microbial respiration and degradation of organic matter by earthworms during the vermicomposting process has been given a significant contribution for the lowering of TOC (Deepthi et al. 2021; Cai et al. 2022).

Significant increase in ash content was observed in final vermicompost of all the BC amended feed mixtures ($p \leq 0.01$). The overall increase in ash content was in the order of 124.69 % in MS_0BC_{10}; 97.29 % in $MS_{25}BC_{10}$; 55.78 % in $MS_{50}BC_{10}$; 36.64 % in $MS_{75}BC_{10}$ and 16.59 % in $MS_{100}BC_{10}$ (Table 2). The percent increase in ash content in all biochar amended treatments was found to be higher as compared to corresponding non biochar treatments. The maximum percent increase was noticed in biochar amended treatment MS_0BC_{10} (124.69) as compared to non biochar treatment MS_0 (105.34). Increment in the ash content was in accordance with many previous studies and has directly been related to degradation and mineralisation of organic matter (Malińska et al. 2016; Ahmed and Deka 2022).The higher increment in biochar amended mixtures could be attributed to enhanced earthworm and microbial activity that resulted in efficient and better mineralisation of feed materials (Paul et al. 2020b).

TKN substantially increased from initial values of 1.24–2.29 % to 2.05–3.38 % in the final vermicompost samples from BC amended feed mixtures ($p \leq 0.05$). The percentage increase in TKN follows the order $MS_{25}BC_{10}$ (73.33 %) > MS_0BC_{10} (66.04 %) > $MS_{50}BC_{10}$ (54.93 %) > $MS_{100}BC_{10}$ (47.96 %) > $MS_{75}BC_{10}$ (41.57 %) (Table 2). Similar observations were made by Khan et al. (2019) who reported the increased levels of nitrogen during the vermicomposting of biochar amended waste. Comparatively, a higher percent increase was observed with biochar amended over non-biochar amended vermicompost samples with a maximum increase in $MS_{25}BC_{10}$ (73.33 %) as compared to MS_{25} (44.16 %). The higher percent increase in biochar amended mixtures could be caused by the increased nitrification due to better environmental conditions for earthworms and nitrifying bacteria facilitated by the addition of biochar (Paul et al. 2020b).

The C/N ratio significantly declined in the final BC amended vermicompost samples ($p \leq 0.05$). The initial values of C/N ranged from 13.64 to 37.50, and by the time vermicomposting process was complete, the values had dropped to 7.92–15.23. The C/N ratio decreased in the sequence: $MS_{25}BC_{10}$ > MS_0BC_{10} > $MS_{50}BC_{10}$ > $MS_{75}BC_{10}$ > $MS_{100}BC_{10}$ (Table 2). Zhang et al. (2016) reported a similar decline in C/N ratio during composting due to BC amendment. All the BC amended vermicompost mixtures showed slightly higher C/N ratios than without BC. These higher values correspond the presence of recalcitrant carbon present in biochar (Khan et al. 2019). The percentage decline in C/N ratio was found to be higher in all the biochar amended vermicompost samples as compared to non biochar vermicompost samples (Table 2).

All the final vermicompost samples had increased TAP levels from BC amended feed mixtures. The greatest increase in TAP was seen in $MS_{25}BC_{10}$ (75.74 %), followed by MS_0BC_{10} (73.55 %), $MS_{50}BC_{10}$ (68.32 %), $MS_{75}BC_{10}$ (31.18 %), and $MS_{100}BC_{10}$ (28.31 %) (Table 2). Among all, only $MS_{75}BC_{10}$ and $MS_{100}BC_{10}$ showed non-significant increase in TAP. BC amended vermicompost samples showed higher increase in TAP when compared to non-biochar vermicompost samples. The increase in $MS_{25}BC_{10}$ vermicompost was 75.74 % while the increase in the corresponding MS_{25} was 58.69 %. These findings were supported by Liu et al. (2021) who reported that the addition of 15 % BC increased the total phosphorus content by 27.3 % as compared to non-BC amended composts (17.2 %). Earthworms and other microorganisms, such as phosphate-solubilizing bacterium and phosphatase enzymatic activity, work together to solubilize biologically bound phosphorus into soluble forms, which increases the amount of TAP in vermicompost (Mago et al. 2022).

The TK concentration ranged from 0.58 to 2.65 % in the initial waste mixtures, and it considerably increased to 0.86–3.44 % after vermicomposting ($p \leq 0.05$). The order of the percent increase in TK was $MS_{75}BC_{10}$ (50.45 %) > $MS_{100}BC_{10}$ (48.26 %) > $MS_{50}BC_{10}$ (42.44 %) > $MS_{25}BC_{10}$(37.20 %) > MS_0BC_{10}(29.69 %) (Table 2). The biochar amended $MS_{25}BC_{10}$ showed 10.83 % higher TK in the final vermicompost compared to non biochar amended MS_{25}.The increment in TK is supported by Liu et al. (2021) and it may have been increased due to the mineralization of organic matter and the loss of biomass (Gusain and Suthar 2020b). Zhang et al. (2016) reported that BC amendment considerably raised the concentration of water-soluble K^+ by 14–59 % as compared to control.

The concentration of TNa enhanced significantly in final vermicompost samples of various feed mixtures ($p \leq 0.05$). The maximum percent increase was observed in $MS_{25}BC_{10}$ (53.39 %) followed by $MS_{75}BC_{10}$ (51.85 %), $MS_{100}BC_{10}$ (50.50 %), $MS_{0}BC_{10}$ (42.03 %), and $MS_{50}BC_{10}$ (39.24 %) (Table 2). Similar increase in TNa was observed by Jain et al. (2018) during the composting of BC amended mixture of organic waste. In the present study, the BC amended mixtures showed significant less TNa than mixtures without BC ($p \leq 0.05$).The biochar amended $MS_{25}BC_{10}$ showed 44.25 % lower TNa content in final vermicompost as compared to non biochar amended MS_{25}. This is a positive development as the higher levels of Na^{+} ions in vermicompost can cause detrimental effects on soil structure (Khan et al. 2019).

3.5 Heavy metal content

HMs are commonly found in compost and vermicompost. So, quantifying the HM content in composts is critical to avoiding soil pollution, metal toxicity to soil organisms, bio-concentration in crops, and meeting regulatory requirements. The heavy metal content in the final product depends upon the presence of heavy metal content in the raw material. Vermicomposting of MS and CD led to increase in the heavy metals (HMs) as compared to their initial values. The increase in the content of heavy metals Cr (15.01–33.33 %), Cu (11.39–43.40 %), Cd (7.81–31.55 %), Mn (12.80–30.34 %), Pb (23.83–58.14 %) and Zn (28.87–80.67 %) was observed in the final vermicompost samples (Table 3). A statistically significant increase was only recorded for Zn and Cd ($p \leq 0.05$). During the vermicomposting, the increment in the heavy metals was reported in the following order: $MS_{75} > MS_{50} > MS_{0} > MS_{25} > MS_{100}$ for Pb; $MS_{25} > MS_{0} > MS_{50} > MS_{75} > MS_{100}$ for Zn; $MS_{75} > MS_{0} > MS_{25} > MS_{100} > MS_{50}$ for Cr; $MS_{0} > MS_{50} > MS_{25} > MS_{75} > MS_{100}$ for Cu; $MS_{100} > MS_{50} > MS_{75} > MS_{25} > MS_{0}$ for Cd; $MS_{0} > MS_{25} > MS_{50} > MS_{100} > MS_{75}$ for Mn (Table 3). Similar to the present study, Gusain and Suthar (2020b) purposed that the increased content of HMs could be due to the mineralisation of organic matter and reduced volume by the action of earthworms that liberated the organically bound metals in the free form. Despite the rise in heavy metals, the concentrations of all HMs in the vermicompost were much lesser than the permissible levels in US composting council and could be utilised as an organic fertiliser in agricultural areas and horticulture (Mohee and Soobhany 2014).

3.6 Effect of biochar on heavy metals

Similar to the feed mixtures without biochar, HMs also increased after vermicomposting of BC amended feed mixtures. The HMs Cr, Cu, Cd, Mn, Pb and Zn showed increments in the range of 25.99–48.87 %, 11.55–71.95 %, 1.72–9.39 %, 7.14–33.24 %, 31.93–61.94 % and 32.45–65.55 % respectively (Table 3). However, among all, only Cr exhibited a statistically significant increase ($p \leq 0.05$). The increment in the heavy metals was reported in the order: $MS_{25}BC_{10} > MS_{75}BC_{10} > MS_{0}BC_{10} > MS_{50}BC_{10} > MS_{100}BC_{10}$ for Pb; $MS_{25}BC_{10} > MS_{50}BC_{10} > MS_{0}BC_{10} > MS_{75}BC_{10} > MS_{100}BC_{10}$ for Zn; $MS_{75}BC_{10} > MS_{50}BC_{10} > MS_{100}BC_{10} > MS_{25}BC_{10} > MS_{0}BC_{10}$ for Cr; $MS_{0}BC_{10} > MS_{25}BC_{10} > MS_{50}BC_{10} > MS_{75}BC_{10} > MS_{100}BC_{10}$ for Cu; $MS_{0}BC_{10} > MS_{100}BC_{10} > MS_{50}BC_{10} > MS_{75}BC_{10} > MS_{25}BC_{10}$ for Cd; $MS_{75}BC_{10} > MS_{25}BC_{10} >$

Table 3
Heavy metal analysis of initial and final vermicompost (Mean ± S.E., n = 3) and percent change over initial (parentheses) in different mixtures of milk sludge and cattle dung with and without biochar.

Heavy Metals (mg/kg)		MS_0	MS_0BC_{10}	MS_{25}	MS_{25} BC_{10}	MS_{50}	MS_{50} BC_{10}	MS_{75}	MS_{75} BC_{10}	MS_{100}	MS_{100} BC_{10}
Pb	Initial	25.12 ± 2.19	18.17 ± 0.96	14.86 ± 0.80	11.15 ± 0.97	10.64 ± 1.15	7.21 ± 0.64	9.85 ± 0.96	5.44 ± 0.52	9.22 ± 0.45	5.37 ± 0.43
	Final	34.15 ± 4.13* (35.93)	25.70 ± 2.70 (41.43)	18.59 ± 1.41 (25.11)	18.06 ± 1.21* (61.94)	16.60 ± 0.83* (56.10)	10.14 ± 0.90* (40.70)	15.58 ± 1.79 (58.14)	8.54 ± 0.64 (56.83)	11.42 ± 1.19 (23.83)	7.08 ± 1.32 (31.93)
Zn	Initial	147.00 ± 4.58	108.33 ± 8.19	210.33 ± 8.99	158.67 ± 11.47	270.00 ± 4.36	190.00 ± 12.50	348.00 ± 10.26	239.00 ± 17.10	426.00 ± 10.58	315.33 ± 9.62
	Final	246.67 ± 11.92* (67.80)	164.67 ± 10.81 (52.00)	380.00 ± 13.32* (80.67)	262.67 ± 10.33** (65.55)	436.67 ± 17.38* (61.73)	295.33 ± 8.09* (55.44)	487.33 ± 11.87* (40.04)	355.67 ± 13.30* (48.81)	549.00 ± 9.85* (28.87)	417.67 ± 8.41* (32.45)
Cr	Initial	31.05 ± 2.10	14.97 ± 2.03	40.10 ± 5.07	24.20 ± 1.14	46.36 ± 0.76	21.01 ± 0.83	47.65 ± 2.75	29.32 ± 1.10	58.20 ± 2.43	36.79 ± 0.90
	Final	40.93 ± 1.78 (31.82)	18.86 ± 2.09** (25.99)	48.37 ± 4.02** (20.62)	33.76 ± 1.80* (39.50)	53.32 ± 1.76 (15.01)	30.13 ± 0.72** (43.40)	63.53 ± 3.02 (33.33)	43.65 ± 0.42** (48.87)	69.49 ± 2.34** (19.41)	52.70 ± 2.64* (43.25)
Cu	Initial	37.53 ± 1.49	24.97 ± 2.42	55.30 ± 1.91	32.20 ± 1.38	59.94 ± 2.38	41.06 ± 0.69	64.16 ± 4.18	49.45 ± 1.90	79.57 ± 2.83	53.70 ± 3.55
	Final	53.82 ± 2.98 (43.40)	42.94 ± 2.06** (71.95)	75.08 ± 1.86* (35.76)	51.38 ± 1.41** (58.97)	81.55 ± 2.92 (36.07)	49.97 ± 2.33 (21.69)	77.93 ± 2.33 (21.46)	56.27 ± 0.92 (13.78)	88.64 ± 2.18* (11.39)	59.90 ± 3.40** (11.55)
Cd	Initial	1.07 ± 0.02	1.10 ± 0.03	1.07 ± 0.02	1.16 ± 0.02	1.13 ± 0.02	1.15 ± 0.01	1.12 ± 0.01	1.24 ± 0.01	1.68 ± 0.04	1.33 ± 0.02
	Final	1.15 ± 0.02** (7.81)	1.20 ± 0.01* (9.39)	1.17 ± 0.02** (9.03)	1.18 ± 0.02 (1.72)	1.24 ± 0.01* (9.73)	1.18 ± 0.02 (2.60)	1.23 ± 0.02* (9.20)	1.26 ± 0.01 (2.16)	2.21 ± 0.06** (31.55)	1.39 ± 0.02* (4.77)
Mn	Initial	178.00 ± 3.61	182.00 ± 8.25	330.00 ± 13.75	249.00 ± 22.81	425.00 ± 14.22	324.00 ± 7.23	500.00 ± 23.39	352.00 ± 12.10	541.00 ± 17.04	408.00 ± 11.72
	Final	232.00 ± 9.29* (30.34)	195.00 ± 7.94 (7.14)	395.00 ± 10.21 (19.70)	328.00 ± 16.20 (31.73)	500.00 ± 23.16* (17.65)	389.00 ± 7.94** (20.06)	564.00 ± 20.75* (12.80)	469.00 ± 10.97* (33.24)	618.00 ± 16.65** (14.23)	528.00 ± 19.22* (29.41)

Student's *t*-test was used to determine the degree of significance (*$p \leq 0.05$), (**$p \leq 0.01$).

$MS_{100}BC_{10} > MS_{50}BC_{10} > MS_0BC_{10}$ for Mn (Table 3). The HMs in non-biochar vermicompost mixtures ranged from 11.42 to 34.15 mg/kg for Pb; 246.67–549 mg/kg for Zn; 40.93–69.49 mg/kg for Cr; 53.82–88.64 mg/kg for Cu; 1.15–2.21 mg/kg for Cd and 232–618 mg/kg for Mn while the BC amended vermin-mixtures showed lower HMs ranging from 7.08 to 25.70 mg/kg for Pd; 164.67–417.67 for Zn; 18.86–52.70 mg/kg for Cr; 42.94–59.90 mg/kg for Cu; 1.18–1.39 mg/kg for Cd and 195–528 mg/kg for Mn. When BC amended vermicompost samples were compared to those without BC, HMs including Cr, Cu, Mn and Zn showed significant lower contents ($p \leq 0.05$). Similar to the present study, lower content of HMs in BC amended treatments have been demonstrated by Khan et al. (2019). This lower content of HMs in BC amended mixtures has been attributed to reduced bioavailability through BC by various mechanisms (precipitation, adsorption, reduction reaction and complexation) (Duan et al. 2021). Moreover, this could also be due to increased earthworm reproduction and growth in biochar amended treatments.

3.7 Structural and maturity analysis of vermicompost

3.7.1 FT-IR

For measuring vermicompost stability and maturity, FT-IR is a crucial instrumental approach. Different band spectra investigations represent the composition of waste feed mixtures, which assists in the detection of shift in functional groups. The pre and post vermicompost FT-IR spectra of MS_{25} and $MS_{25}BC_{10}$ are expressed (see Supplementary material). The FT-IR data of MS_{25} and $MS_{25}BC_{10}$ original raw material and final vermicompost is construed based on references Singh et al. (2022), Quadar et al. (2022) and Mago et al. (2022). The spectra of FT-IR observed in initial MS_{25} had varied peaks at around 3323 cm^{-1} (–OH, –COOH group and ROH), 2920 cm^{-1} (–CH stretch of aliphatic CH_2), 1637 cm^{-1} (–CO, RCOOR, NH_2, and CHO),1031 cm^{-1} (–CO stretch of polysaccharides and cellulosic compounds). The FTIR spectra of initial biochar amended $MS_{25}BC_{10}$ had shown peaks near to 3367 cm^{-1} (–OH, –COOH and ROH), 1597 cm^{-1} (–CO, RCOOR, NH_2, and CHO), 1386 cm^{-1} (–NO stretch of NO^{3-}) and 1033 cm^{-1} (–CO cellulosic and polysaccharides). Comparing the post vermicompost sample of MS_{25} to pre vermicompost sample, a reduction in peak intensities with broadness was observed around 3323 cm^{-1} (for carboxylic, phenols and alcohols groups) which is likely resulted from the biodegradation of lignin and amines, corroborating the stability of vermicompost. The decline of CH_2 and CH_3 groups suggesting the breakdown of aliphatic compounds is responsible for the decrease in peak intensities at 2920 cm^{-1}. The reduction in peak intensities at 1637 cm^{-1} supports breakdown of complex molecules in the final vermicompost, like aromatic rings of ketones, amides, carboxylic acids, and aromatic molecules, $C{=}C$, $N{-}O$ and $C{=}O$ stretching vibrations in NO^{3-} and $-NH_2$, in the resultant vermicompost. The alterations in polysaccharide breakdown resulted into declined peak intensity of final MS_{25} vermicompost near at 1031 cm^{-1} (Mago et al. 2022).

Comparing the post vermicompost of $MS_{25}BC_{10}$ to pre vermicompost samples a reduction in peak intensities with broadness was observed around 3367 cm^{-1} (for carboxylic, phenols and alcohols groups) which is likely resulted from the biodegradation of lignin and amines during the process of vermicomposting, leading to stability of vermicompost. The decline in peak intensities at 1597 and 1386 cm^{-1} supports breakdown of compounds in final vermicompost, *viz.,* aromatic rings of ketones and amides, carboxylic acids, and aromatic molecules, $C{=}C$, $N{-}O$ and $C{=}O$ stretching vibrations in NO^{3-} and $-NH_2$, in the resultant vermicompost. The alterations in polysaccharide breakdown resulted in declined peak intensity of $MS_{25}BC_{10}$ (final) vermicompost near at 1033 cm^{-1} (Singh et al. 2022).

3.7.2 Scanning electron microscopy (SEM)

SEM gives critical information about surface profile and allows us for a comparison of the feed mixtures before and after vermicomposting. The surface morphology of pre and post MS_{25} and $MS_{25}BC_{10}$ vermicompost samples were analysed employing SEM. The SEM scans of the initial waste mixture indicated compact mass that showed lack of pores and fragmentation (see Supplementary material). After vermicomposting, the end products were converted into porous, fragmented and disaggregated structures. Earthworms utilized and shattered the waste mixtures by grinding action that resulted in vermicompost fragmentation and disintegration, as shown by superficial changes in the vermicompost samples (see Supplementary material). The SEM analysis results were in accordance to Quadar et al. (2022) demonstrating a change in initial substrate morphology following vermicomposting. Recently, Singh et al. (2022) demonstrated that joint action of earthworms and microbes produced surface coarseness, irregularities and fragmentation in vermicompost that produced better quality manure during the vermicomposting of allopathic pharmaceutical industry sludge.

3.7.3 Seed germination index (GI) for vermicompost maturity

The seed germination bioassay (GI) is reliable biological test system used to assess the vermicompost toxicity and level of maturity. The GI value of > 80 % demonstrate the maturity and non-phytotoxicity of the compost (Singh et al. 2022). Different types of seeds have been tested for GI of various vermicompost including 235–380 % for maize and 228–388 % for chick pea (Quadar et al. 2022) and 175.9–155.02 % for *Vigna radiata* (Singh et al. 2022). In the present study, the different feed mixtures showed significant difference in the GI of the final vermicompost samples ($p \leq 0.05$). The maximum GI was observed in MS_{25} (131.46 %) followed by MS_0 (110.77 %), MS_{50} (106.75 %), MS_{75} (92.02 %) and MS_{100} (89.14 %). On the other hand, the sequence of GI in BC amended mixtures followed the order $MS_{25}BC_{10}$ (153.47 %) followed by MS_0BC_{10} (142.45 %), $MS_{50}BC_{10}$ (135.33 %), $MS_{75}BC_{10}$ (121.15 %) and $MS_{100}BC_{10}$ (115.18 %) (Fig. 2). GI values in the final vermicompost's were>80 %, which indicated that vermicompost is mature and has no phytotoxicity. The BC amended mixtures showed significantly increased GI values compared to the mixtures without BC ($p \leq 0.05$). This higher GI values could be linked to the capacity of BC to captivate phytotoxins (Wang et al. 2017). The higher GI value in the BC amended mixtures indicates the higher degree of maturity. Wang et al. (2017) also observed that the treatments amended with BC showed higher GI when compared to treatment without BC during the composting of beer vinasse (GI of 142.1 % for 15 % BC and 120.9 % without BC).

4. Conclusion

The present study revealed the potential of vermicomposting and the synergistic effect of biochar amendment in vermicomposting of milk industry sludge. The mixtures, MS_{25} and $MS_{25}BC_{10}$ feed treatment produced minimum mortality and efficient development for *E. fetida*. The BC amended feed mixtures showed significantly high cocoon and hatchlings development. The addition of BC also led to enhanced nutrient recovery, maturity, reduced phytotoxicity and significantly lower HM content than mixtures without BC. Thus, BC amendment further results in enhanced mineralisation, increased nutrient content and reduced content of heavy metals producing high quality vermicompost for agricultural applications.

CRediT authorship contribution statement

Rahil Dutta: Investigation, Conceptualization, Writing - original draft, Writing - review & editing. **Deachen Angmo:** Formal analysis. **Jaswinder Singh:** Resources, Supervision, Writing - review & editing. **Anu Bala Chowdhary:** Formal analysis. **Jahangeer Quadar:** Formal analysis. **Sharanpreet Singh:** Data curation. **Adarsh Pal Vig:** Conceptualization, Resources, Writing – review & editing, Supervision.

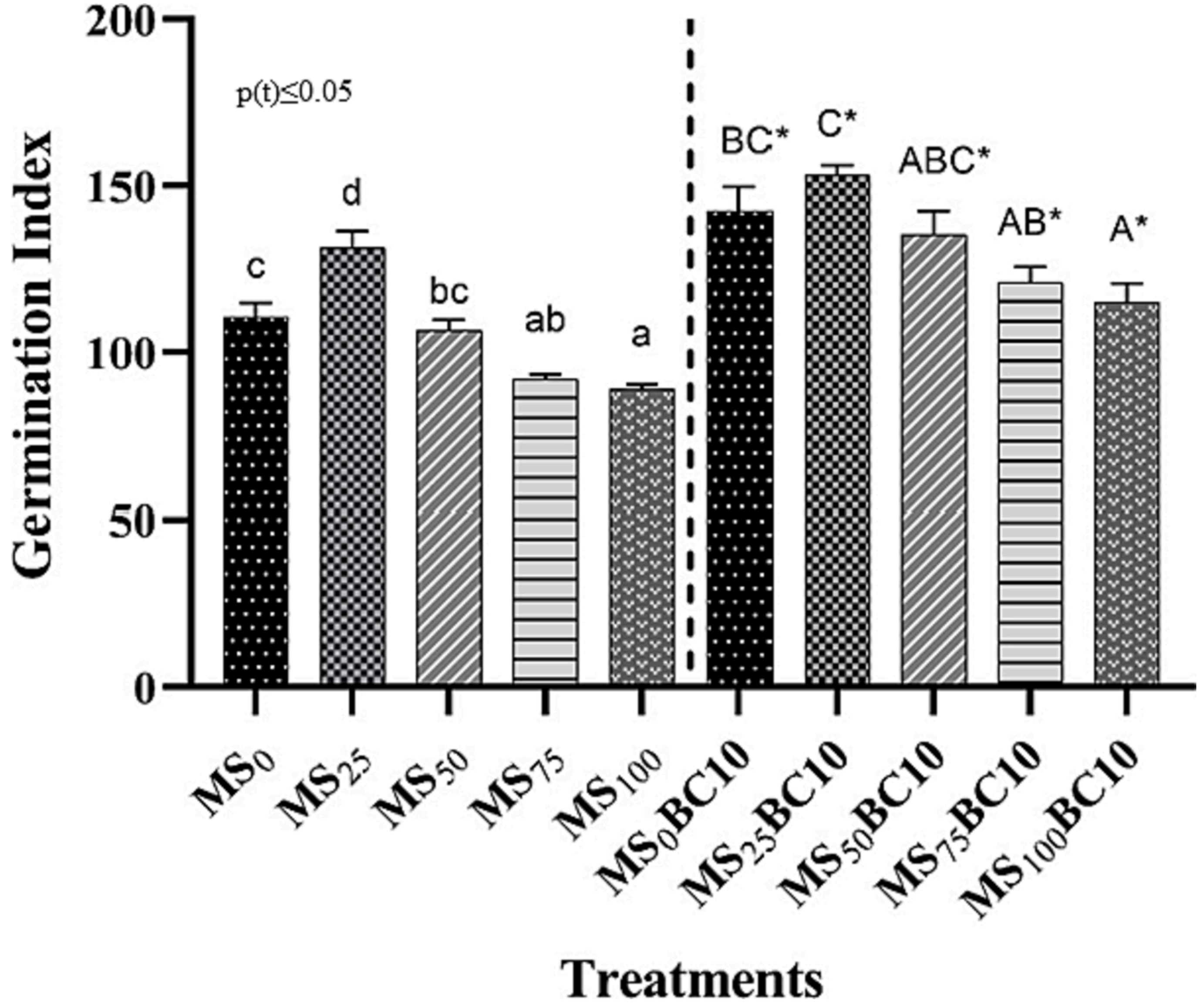

Fig. 2. Germination Index (GI) for final vermicompost of different feed mixtures. Small letters and capital letters denote significant differences among feed mixtures without biochar and with biochar respectively at ($P \leq 0.05$) calculated through Tukey's test. * Indicate the significant difference ($P \leq 0.05$) between the treatments without biochar and with biochar calculated by independent *t*-test.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

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Appendix A. Supplementary data

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