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ORIGINAL ARTICLE

Bioconversion of biochar-supplemented pressmud into enriched vermicompost employing *Eudrilus eugeniae*

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Abstract

Vermicomposting is a method to decompose complex organic materials into simpler humus compounds thereby restoring soil fertility. Sugarcane industries release different waste products such as bagasse, pressmud and molasses in the processing of sugarcane which may lead to environmental pollution. The pressmud can be converted into useful manure with high nutrients for agricultural purposes. In this study, the pressmud is converted into enriched vermicompost supplemented with biochar. This study was divided into 12 combinations with pressmud and cow dung at different compositions and supplemented with biochar in varied concentrations of 0, 2, 4 and 6% by employing *Eudrilus eugeniae* for the production of vermicompost. The combinations supplemented with biochar were shown to possess a higher level of physiochemical parameters and nutrient contents. The level of pH, C/N ratio, C/P ratio and total organic carbon displayed a significant decrease in the final vermicompost compared to the initial sample. The highest decrease was noted in 2% and 4% biochar-supplemented groups. The level of macro and micronutrients such as sodium, potassium and ferrous were higher in the groups integrated with biochar. The electrical conductivity, total nitrogen, phosphorus and potassium levels were increased in the final vermicompost groups with 1:1 and 2:1 composition of pressmud and cow dung supplemented with biochar with the maximum increase at 4% biochar amendment. The FTIR and SEM analysis confirmed the decomposition of the final vermicompost groups amended with biochar. The final nutrient-enriched vermicompost can be produced from pressmud and cow dung at a 1:1 and 2:1 ratio supplementing with 4% biochar. The amendment of biochar with pressmud improved the quality of vermicompost by maintaining the physico-chemical parameters at the ideal level and retaining the nutrient levels.

Keywords Biochar · Eudriluseugeniae · Sugar industry waste · Vermicomposting

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1 Introduction

Sugarcane (*Saccharum* sp.) is a significant industrial crop cultivated in more than 80 tropical and sub-tropical nations [1]. It is a perennial grass, a fundamental raw material in sugar production [2]. The world's biggest consumer and the second biggest producer of Sugarcane is India after Brazil [3]. It yields approximately 362 Mt of cane annually and contributes approximately 15% to world sugar production [4]. India has 732sugar mills, producing an estimated 3.5×10^9 kg of sugar, making it a momentous agro-based sector in its economy [5]. According to Chakraborty et al., [6], Indian sugar production is focused in nine large states, led by Maharashtra with 27% and Uttar Pradesh with 30% of total production. Andhra Pradesh, Gujarat, Karnataka Tamil Nadu, Bihar, Punjab, and Haryana are the other important

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producers contributing to 94% of India's total sugar production. A sugar mill generates waste products such as press mud, molasses, bagasse, and other materials in addition to sugar [7]. The press mud or filter cake is generated after the distillation of sugar from the sugarcane juice. Usually, 100 tons of cane trash yield around 3% press mud cake and is composted to yield highly nutrient-rich organic fertilizer [8]. Press mud is a soft, spongy, amorphous and dark brown in nature material containing sugar, fibre and coagulated colloids including cane wax, albuminoids, inorganic salts and soil particles [9]. Press mud has the potential to drastically boost the production of crops and soil properties [10]. Press mud renders as an ideal fertilizer due to the richness of organic carbon, sugar, protein, enzymes, organic matter, micro and macro nutrients [11].

Dotaniya et al. [10], observed that sugarcane produces high amounts of by product which require storage and disposal facilities in India and their safe and sustainable handling is a persistent concern for environmentalists, policymakers, and scientists. Bhat et al. [12] pointed out that the open dumping of these agricultural waste leads to the pollution of the environment and carries major health problems to humans. Based on numerous reviews, vermicomposting of pressmud is rated one of the most prospective technologies for solid waste treatment. By composting, organic substrates in solid waste can be stabilized and biodegraded and result in a final compost product that can serve as a soil conditioner or fertilizer.

Lim et al., [13] analysed that earthworms speed up decomposition of organic matter by breaking down and oxygenating waste, significantly reducing compostingtime compared to traditional composting practices. Moreover, the humus content of vermicompost enhances soil porosity, aeration, and water holding capacity, rendering it a better soil conditioner than traditional compost [14]. Vermicompost is produced by the interaction of earthworms and microbes which together breakdown and digest waste components into a finer, humified and microbially active material [15]. It is an environmentally friendly method, which can disintegrate organic waste as well as inorganic waste in soils [16, 17]. In accordance with Singh et al. [18], it is a widely recognized and cost-effective method to enhance the fertility of soil by employing earthworms as vermicomposting agent which can act as soil enhancers in agriculture.

The choice of an appropriate earthworm species is important in vermicomposting. Out of the 4,000 known earthworm species, belonging to endogeic, anecic, and epigeic species, only some of the epigeic species are used for vermicomposting [19]. Thriving in subtropical and tropical climates, *Eudrilus eugeniae* has been a useful agent in vermicomposting, readily converting varied crop residues into nutrient dense compost which is reported in experiments [11, 20, 21]. *Eudrilus eugeniae* provides greater vermicompost yield within a shorter time and facilitates fast reproduction, improving decomposition [22]. Hence, *Eudrilus eugeniae* or African night crawler was used in the current study.

The addition of additives to composting is an inventive approach towards the reduction of environmental pollution with the acceleration of decomposition [23]. Of these, biochar has recently gained strong attention as an effective stabilizer for biowaste [24]. Its advantages in composting varying from enhanced microbial activity to better retention of nutrients are well established in many studies [25, 24, 26, 27]. Furthermore, the amendment of biochar with vermicompost facilitates more effective breakdown of hazardous substances because earthworm and biochar collectively sequester heavy metals [28, 29]. The unique properties of biochar includes high porosity, sorption capacity, cation exchange capacity and chemical recalcitrance, have gained consideration [30]. Biochar's porous attribute optimizes the porosity of compost, which in turn enhances optimal oxygen supply and averts anaerobic respiration [26]. The earthworm's extracellular enzymes trigger the biochar to become bioactive [31]. In the current research, the vermicompost has been treated with biochar to assess its efficiency.

This research proposes an alternative method of managing waste from the sugarcane industry through the incorporation of biochar in vermicomposting. This research assesses the physicochemical properties, SEM, and FTIR characterization of organic substrates with various concentrations of biochar (2%, 4%, 6%) in cow dung-press mud mixtures, and vermicompost, which are used to evaluate the nutrient contents' change in the end product. The results provide a sustainable substitute to chemical fertilizers, enhance organic recycling of wastes, and aid in the conservation of the environment.This study bridges the gap by determining the best proportion of biochar in vermicomposting for enhanced decomposition, and quality of compost for recycling waste sustainably. Moreover, the outcomes have general applicability to the management of agro-industrial waste, sustainable agriculture, and commercialization possibilities of biochar-amended vermicompost products.

2 Materials and methods

2.1 Collection of raw materials

The main raw material press mud was obtained from Kallakurichi Co-operative sugar mill at Moongilthuraipattu, Tiruvannamalai, Tamil Nadu. Earthworm species *Eudrilus eugeniae* was chosen for this research on the basis of its unique physical features. They were identified through their reddish-brown coloration on the dorsal side, smooth texture, and rapid movement. They ranged in length from 15 to 25 cm with a clearly demarcated clitellum found on segments 13–17 and weighing between 0.6-1gm.It was collected from VST natural farm, Kundumaranapalli, Hosur, Tamil Nadu. Urine- free fresh cow dung was procured nearby farm-house, Chinnaleasagiri, Hosur. The supplementary substrate, biochar obtained from Greenfield Eco solutions.

2.2 Precomposting of pressmud

Precomposting is an important pretreatment step that enables raw waste materials to aerobically decompose prior to subsequent composting. For the study, the pressmud was spread to a pristine floor over the partial shade of sunlight for a period of five days to remove the noxious gases and extra moisture content. Following the initial drying process, the pressmud was then shifted into compost bags for controlled precomposting. To ensure there was adequate moisture to support microbial decomposition, water was sprinkled over the material on a periodic basis. The precomposting period took 30 days, during which necessary nutrients were rendered bioavailable and the substrate became more appropriate for vermicomposting. It ensures that when earthworms are added, they can efficiently decompose organic matter. Studies of Karmegam et al. [32] indicates that precomposting increases the efficiency of vermicomposting by promoting microbial colonization and improving the quality of the final compost.

2.3 Vermicomposting of substrate

For the study, twelve different combinations of substrate (C1 to C12) with pressmud, cow dung and biochar were used. 5 Kg capacity of circular grow bag was utilized to fill the substrate. Two Kilograms of substrate material were kept in triplicate based on dry weight for the vermicomposting process. The twelve-vermicomposting bags were made from pre-composted pressmud and cow manure at various proportions, which had been amended with different combinations of biochar. Randomly chosen twelve clitellated earthworms, *Eudrilus eugeniae* and were introduced in each vermicomposting bag [33]. For effective vermicomposting, a humid, dark, undisturbed environment was maintained for the entire vermicomposting bag at a temperature of 25± 3 °C.During the vermicomposting process, the moisture content was kept at 60–70% by regular irrigation. The vermicomposting bags were covered with cotton cloth to prevent moisture loss and to protect earthworm from predators. The experimental setup was maintained for 75 days, and samples were taken for analysis at regular intervals of fifteen days. In the present work, the controlled moisture and proper aeration prevented the leaching process. The vermicompost was air-dried, sieved, and stored for further examination and assessment.

Combinations were made to evaluate the effects of varying pressmud-to-cow dung ratios and supplementation with biochar on vermicomposting. Pressmud contributes organic carbon, and cow dung contributes nitrogen, maintaining an optimal C: N ratio for microbial and earthworm growth. Biochar (2%, 4%, 6%) was supplemented to test its function in aeration, nutrient immobilization, and heavy metal sequestration. Control sets (without biochar) aid in comparison. The stepwise biochar increase guarantees optimal dosage evaluation, determining conditions that promote earthworm activity and compost quality (Table 1).

2.4 Characterization and analysis of vermicompost

The physicochemical analysis of the vermicompost was carried out, which was produced with different combinations of pressumd+cowdung along with or without biochar. The Jackson [34] method was implemented to ascertain the pH and EC of the material using 1:10 ratio of compost with distilled water. The pH meter with combined electrode (Elico model LI-120 pH metre) was used to calculate the pH. The Jackson [34] method was used for evaluating the compost's electrical conductivity by using the Conductivity Bridge (Type CM-82T). The total organic carbon was determined employing the Walkley and Black procedure [35]. The sample was oxidized with chromic acid, and the excess dichromate was titrated with ferrous ammonium sulphate by using diphenylamine as an indicator. Organic carbon is computed as 10 - (10 × y/x) ml of 1 N potassium dichromate, where x represents the volume of the blank titration and y the sample. Total nitrogen was analysed by the Micro-Kjeldahl method [36]. The sample

Table 1 Vermibed combinations with pressmud, cowdung and biochar

Combinations	Composition (2 kg/vermibed)		Supplementary substrate composition (Biochar, %)
	Ratio of pressmud	Ratio of cowdung	
C1	1	1	-
C2	1	1	2
C3	1	1	4
C4	1	1	6
C5	2	1	-
C6	2	1	2
C7	2	1	4
C8	2	1	6
C9	3	1	-
C10	3	1	2
C11	3	1	4
C12	3	1	6

was digested with sulfuric-salicylic acid, sodium thiosulfate, and a mixture of sulphate, followed by distillation in a micro-Kjeldahl apparatus. The digest was treated with boric acid and NaOH, and the distillate was titrated with N/200 H_2SO_4. Total nitrogen was calculated as N (%) = TV × 0.28, where TV is the sample minus blank titration value.

Phosphorus (P) from manure was determined by diacid digestion procedure [36]. A 1 g powder sample was digested in 9:4 HNO_3: $HClO_4$, heated to stop NO_2 fumes, and evaporated to 3–5 ml without drying. The solution, after cooling, was made up to 20 ml with deionized water and filtered. Phosphorus was colorimetrically measured by ammonium molybdate-ammonium vanadate reagent in nitric acid. The calibration curve was plotted using 50 ppm KH_2PO_4 standard solution. Standard solutions (0–5 ppm P) were treated, incubated for 30 min, and read at 420 nm for absorbance. Sample P content was calculated based on the comparison of absorbance with the standard curve.Potassium (K) was determined by flame photometry [36]. A stock solution of 1000 ppm K was made with 1.91 g KCl/L, and standards (10–50 ppm) were prepared by dilution. The CL-22D flame photometer was calibrated by running distilled water (zero) and 50 ppm K solution (100). The readings of the standards were plotted to obtain a calibration curve. The sample was atomized and analyzed for K content from the curve, and it was then multiplied by the factor of dilution.

Sodium (Na) was calculated by flame photometry [36], as for potassium. The 1000 ppm Na stock solution was prepared from 2.54 g NaCl/L, and standards (5–25 ppm) were prepared through dilution. A standard curve was created, and the Na content of the sample was assayed using a flame photometer.Calcium and magnesium were determined according to Tandon ,1993 [36] by EDTA titration. A standard of 1000 ppm of calcium was obtained from $CaCO_3$. EDTA solution was standardized by titration with the calcium solution with the required buffers and indicators. For the determination of calcium, the aliquot of sample was treated with NaOH and a calcium indicator and titrated with EDTA to a blue endpoint. For total Ca + Mg, the same procedure was repeated using ammonium chloride-hydroxide buffer and Erichrome Black T (EBT) indicator, after warming. Magnesium was calculated as Ca + Mg minus Ca. The Ca/Mg ratio was estimated from their milliequivalents and equivalent weights.

Sulphur content was estimated through barium sulphate turbidimetry [36] following wet ashing and diacid digestion. Analytical grade reagents were barium chloride, gum acacia, salt buffer ($HgCl_2 \cdot 6\ H_2O$, KNO_3, ethanol), 6 N HCl, and a 100-ppm sulphur standard (K_2SO_4). Sulphur standards (0–20 ppm) were prepared and determined at 420 nm using a Model CL-27 spectrophotometer. The same treatment was given to a 10 ml aliquot of the digest of the sample and sulphur content was calculated from the standard curve. Zinc and copper contents were approximated by Atomic Absorption Spectrophotometry (AAS) [36]. Zinc (1000 ppm) and copper (1000 ppm) standard solutions were made by dissolving Zn and Cu metals in (1 + 1) HCl and HNO_3 respectively and diluting with 1% acid. Working standards were made, and a standard curve was plotted with the help of four different concentrations and a blank. Samples were measured, and Zn/Cu concentrations were computed based on absorbance values. Fe content was approximated with a CL-27 spectrophotometer [34]. A stock solution of 100 ppm Fe was made from ferrous ammonium sulphate in 0.6 N HCl. Working standards were diluted, and a standard curve was plotted. To analyse, an aliquot of a digested sample (5–60 µg Fe) was brought to pH 1.5–2.7, treated with hydroxylamine hydrochloride and ortho-phenanthroline, diluted to 25 ml, and its absorbance measured. Fe concentration was calculated from the standard curve.

Manganese (Mn) concentration was estimated on a CL-27 spectrophotometer [37]. A stock solution of 1000 ppm Mn was made by dissolving Mn metal in (1 + 1) HNO_3 and making up to volume with 1% HCl. The standard Mn solutions were diluted, and a standard curve was plotted. For the samples, a dry-ashed solution was digested with HNO_3, H_2O_2, and phosphoric acid and heated. On cooling, water and potassium periodate were added, and it was heated to pink. The absorbance at 540 nm was taken, and Mn concentration was calculated standard graph.

The percentage of carbon to the percentage of nitrogen i.e., C.N ratio was determined by dividing the estimated percentage of carbon for the sample by the percentage of nitrogen estimated for the sample. The ratio of the percentage of carbon to that of phosphorous i.e., C/P ratio was determined by dividing the percentage of carbon estimated for the sample by the percentage of phosphorus estimated for the same sample in the current study. The percentage increase/decrease of different chemical parameters over the worm-unworked substrates was determined [38].

2.5 FTIR spectroscopy analysis

In this research, Fourier Transform Infrared (FTIR) Spectroscopy was employed to analyze the biochemical changes in vermicompost from press mud, both amended and unamended with biochar. This robust analytical tool is capable of disentangling the complex mechanism of mineralization, elucidating the degradation of raw materials during vermicomposting. By indicating the presence of important functional groups, FTIR offers significant information regarding the chemical stability and maturity of the end compost, and its appropriateness for use in agriculture [12].

For sample preparation, one milligram of vermicompost was carefully mixed with one hundred milligrams of potassium bromide (KBr), a clear medium suitable for infrared studies. This blend was subsequently exposed to one megapascal (MPa) pressure, resulting in dense granules about 10 mm in diameter and 1 mm thick [39]. The compost was scanned between a wavelength range of 4000 to 400 cm^{-1} using the FTIR Spectrum 100 (Perkin Elmer, USA), picking up the distinct infrared signatures that identify the complex biochemical transformations that take place in the vermicomposting process.

2.6 Scanning electron microscopy (SEM) analysis

Scanning Electron Microscopy (SEM) was used to examine both initial and finalday vermicompost samples. SEM gave a clear image of the surface morphology, showing the texture and structural changes as organic matter was degraded by microbial and earthworm action. Proper sample preparation was necessary before imaging to produce high-quality results. To remove the moisture content, the samples were dried at 70 ± 2 °C with caution until they reached constant weight to avoid any microstructural distortions. The dried samples were then firmly mounted on a metallic holder utilizing double-sided adhesive carbon tape for stability during the imaging process. For the purpose of improving image quality and conductivity, a thin layer of gold coating was deposited on the sputter coater to enable a clear and well-defined visualization of the microstructures of the compost. Images were taken at different magnifications using the IEOL JM-5600 electron microscope, revealing the intricate and dynamic structure of the vermicompost. High-resolution images revealed information on particle aggregation, porosity, and microbial colonization, highlighting the extensive changes taking place at a microscopic level during the composting process.

2.7 Statistical analysis

The earthworm growth parameters and vermicompostchemical results have been assessed and presented as mean± standard deviations, based on the average of three replicates. Applying Analysis of Variance (SPSS software) at the level of 5% significance between the initial and final values of each treatment parameter for chemical variables recorded on 0, 15,30,45,60 and 75 days were evaluated.

3 Results and discussion

The pH, Electrical conductivity (EC), Total Organic carbon (TOC), Total Kjeldahl Nitrogen (TKN), Total Phosphorus (TP), Total Potassium (TK), C/N ratio (Carbon/Nitrogen) and C/P ratio (Carbon/ Phosphorus) of the vermicompost was measured using compost samples collected from pressmudat various study stages (0, 15, 30,45,60, and 75 days) (Figs. 1, 2, and 3) and the initial (0 day) and final (75th day) data in Table 2. The two key metrics for evaluating the maturity and quality of vermicompost are pH and EC values. In the present study, pressmud pH reduced from initial to final stage with the minimum final pH obtained was 6.81 in C7 and the maximum was 7.93 in C9. Earlier literature indicates that the process of bioconversion results in the generation of carbon dioxide, ammonia, nitrates, orthophosphates, and organic acids, which are responsible for the decrease in pH [40, 41, 42, 32]. Adding cattle dung to different organic substrates increases the activity and growth of microorganisms and earthworms, which in turn produce organic acid and by-productsthat can alter the pH of the final vermicompost [43, 44]. The final pH of this study varied between 6.80 and 8.20, which allows plants to access different soil nutrients.Lower biochar concentrations have been shown to optimize pH stabilization by research, with over-application potentially resulting in too high a pH that discourages microbial and earthworm activity [45].

The salinity of the organic material in the vermicompost is determined by EC. According to studies [46, 47, 48], the production of inorganic ions and dissolved substances like phosphate, ammonium and nitrate may increase in EC in vermicompost. This shows that vermicomposting promotes the mineralization of organic matter by converting insoluble material into soluble one. From the initial to final feed, the current study displays a progressive rise in electrical conductivity. In C8, the highest value obtained was 1.32, and the lowest was 0.78 in C9. The electrical conductivity value in the present study varied from 0.74 to 1.32. So, this proves that, the vermicompost amended with biochar can be a safe supplement to the soil.

Carbon is a key component of organic molecule, which are the building units of all living things and they became the energy source for the composting process [49, 50]. Various physical, chemical, biological reactions occur during the vermicomposting process and leads to the transformation of organic matter. Vermicomposting reduces total organic carbon through microbial respiration and earthworm activity [51, 52]. According to Khatua et al. [53] and Yang et al. [54] that the observed decrease in total organic carbon indicates that microbial activity in the compost lead to the biodegradation and mineralization of organic compound result in the release of carbon dioxide. In the present study showed a steady decrease in total organic carbon from initial to final feed. The highest reduction was observed in C3 at 27.97, while the lowest reduced final value recorded was 42.58 in C12.

Among all the treatments, C6 and C7 showed significant ($P < 0.05$) change in the pH from the initial level to final

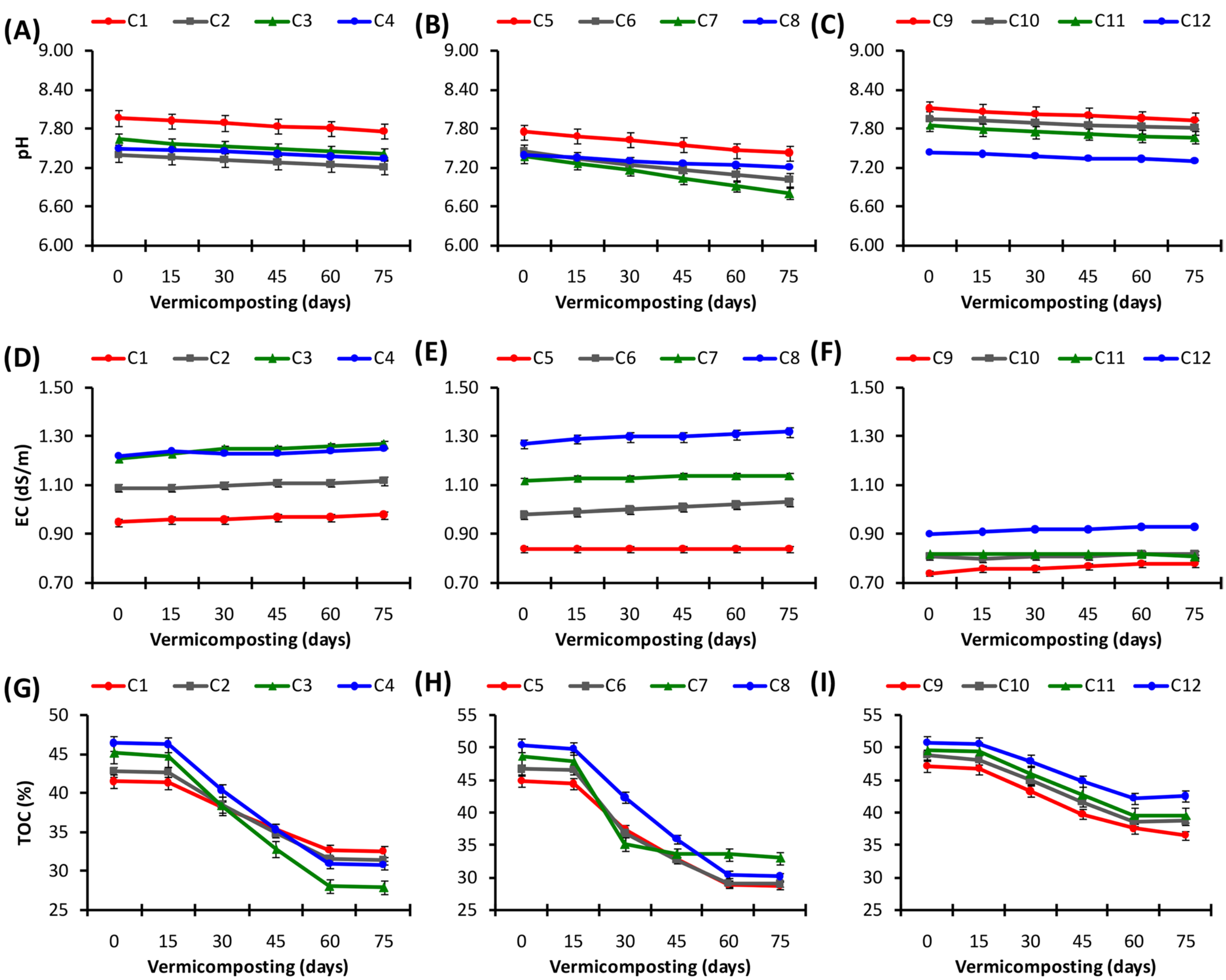

Fig. 1 The changes in pH **A**–**C**, EC **D**–**F**, and TOC **G**–**I** contents during vermicomposting of pressmud + cow dung substrates amended with different proportions of biochar. Values are mean ± standard deviation (error bars)

level while the increase of EC values in C6 alone was found to be significant ($P < 0.05$). Irrespective of the treatmemnts, TKN, TK, and TP showed significant incremental change whereas, TOC, C/N ratio and C/P ratio showed significant decrease. The percentage change of TKN, TK, TP, TOC, C/N ratio and C/P ratio in C7 recorded maximum in C7 treatment followed by C8 treatment (Table 2). Jindo et al. [25] observed that biochar improved aeration and microbial diversity, which contributed to enhanced organic carbon degradation. In contrast, excessive biochar application may lead to a reduced decomposition rate due to microbial inhibition and carbon stabilization [55]. Therefore, the lower percentage of organic carbon in the present study indicate that the earthworm accelerated the decomposition of organic matter in the pressmud.

Earthworm contribute nitrogen content in the vermicompost by decomposing dead earthworm tissues and microbial mediated nitrogen transformation result in an additional increase of nitrogen as per the context of Suthar [56]. In accordance with Kaushik and Garg [57] and Suthar and Singh [58] that the decomposition and mineralization of the organic substrate as well as the conversion of NH_4 to NH_3, were the reasons for the higher trend in nitrogen in the vermicompost processed by earthworm. In the present study, nitrogen exhibits the level of increase from the initial to the final day. The highest increase was observed in C7 at 2.62, while the lowest increase was recorded in C1 at 1.08. The findings of Cynthia and Rajesh Kumar [59] that the decomposition of waste by worms to accelerate the nitrogen mineralization process is the cause of rise in nitrogen content of sugar mill effluent. But excessive use of biochar could immobilize nitrogen and retard mineralization, affecting total nitrogen concentration based on the properties of biochar and dosages [60]. In this study, vermicomposting with

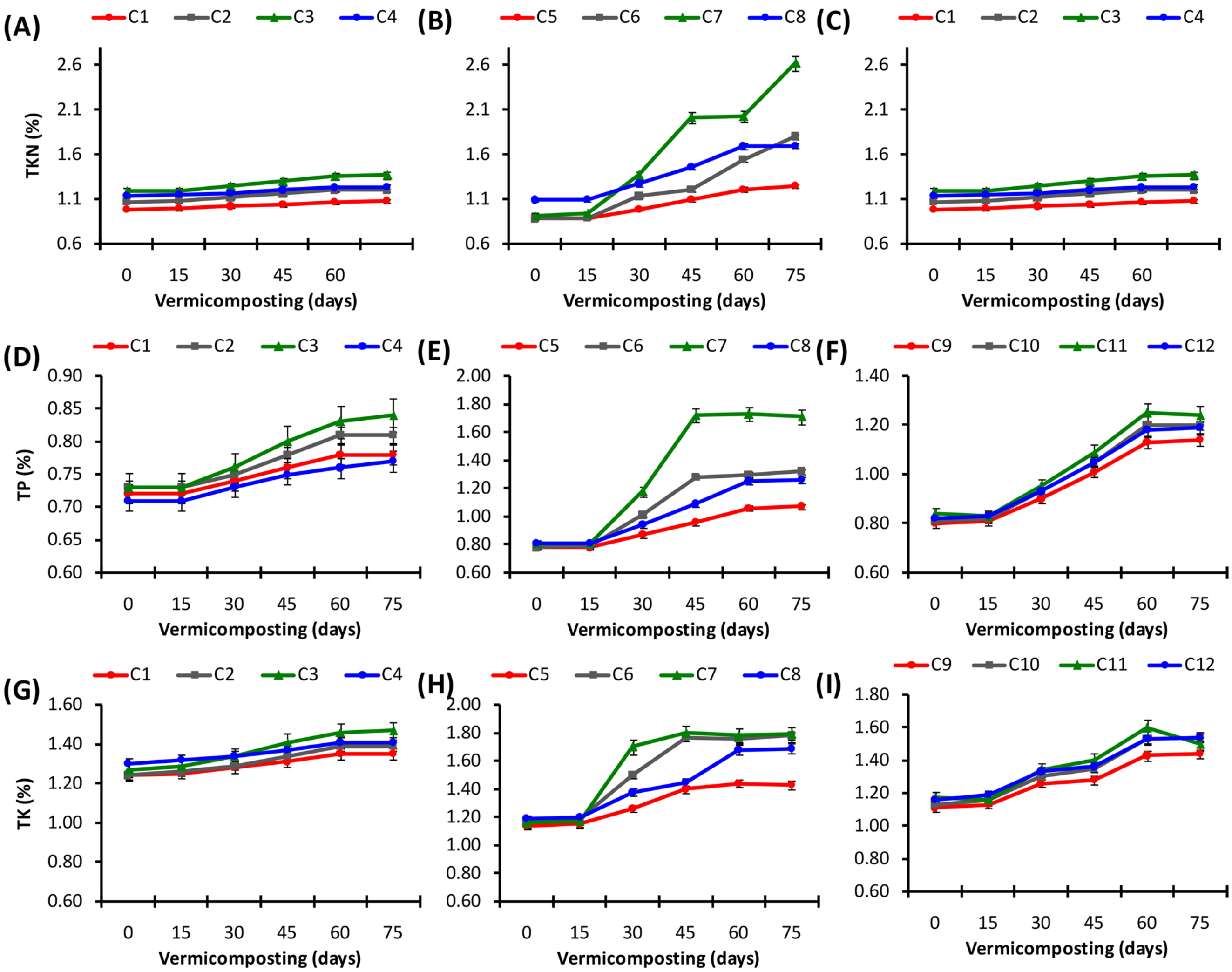

Fig. 2 The changes in TKN **A**–**C**, TP **D**–**F**, and TK **G**–**I** contents during vermicomposting of pressmud + cow dung substrates amended with different proportions of biochar. Values are mean ± standard deviation (error bars)

biochar supplementation led to higher total nitrogen content, which indicates that biochar was involved in the conservation of nitrogen and promoted the activity of microbes during the decomposition of press mud.

Yadav and Garg [61] suggested that the increase in phosphorous content may have been caused by mineralization and mobilization of organic matter by earthworm, as well as by the interaction of microbes and phosphate excretion. In the present study the level of total phosphorous increases from the initial day to final day in almost all combinations. However, the combinations of C1, C2, C10 showed gradual increase of total phosphorous till 60 days and maintained same percentage at 75th day. The other exception is that C11 showed reduction from 60th day to 75th day. Parveresh [62] reported a decrease in phosphorous level, which was related to the adsorption of inorganic phosphorous, released from the earthworm's tissues. Eariler studies have revealed that the addition of biochar can enhance total phosphorus content in compost [25, 56, 27]. Biochar-amended vermicomposting in the current study resulted in the elevation of phosphorus content, reflecting its capability to enhance phosphorus stabilization as well as microbial activity in press mud decomposition.

According to Garg et al. [63] that the level of potassium increases due to acid production by microbes, which caused the solubilization of organically bound potassium. In the present study, most of the combinations showed a progressive increase of total potassium from initial day to final day. Some combination exhibits an increase of potassium content from initial day to till 60th day, with the same percentage maintained at 75th day. The highest increase in potassium was observed in C7 at 1.79, while the lowest was recorded in C9 at 1.35. Variation in total potassium concentration among the combinations is due to the variation in the chemical composition of initial waste. The findings of Khatua et al. [53] and Pramanik et al. [64] that an earthworm's gut can

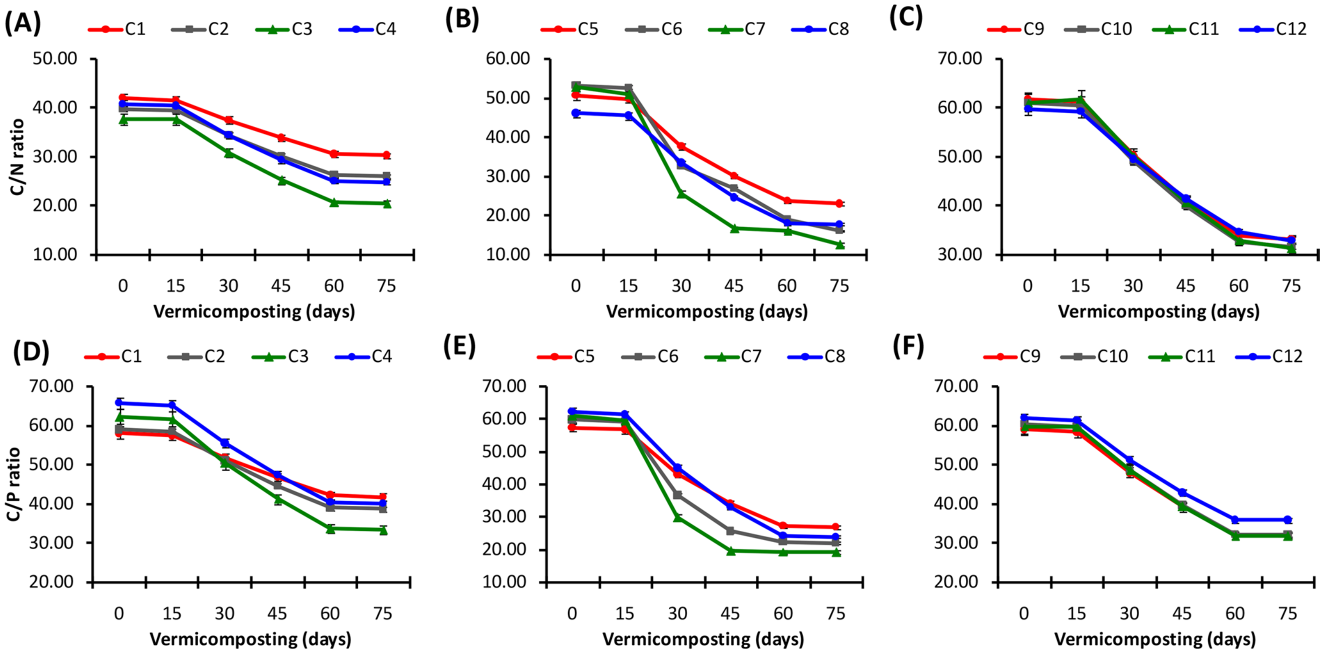

Fig. 3 The changes in C/N ratio **A**–**C** and C/P ratio **D**–**F** during vermicomposting of pressmud + cow dung substrates amended with different proportions of biochar. Values are mean ± standard deviation (error bars)

increase the release of potassium in vermicompost. Huang et al. [65] found that biochar enhanced the solubility and mobility of potassium, resulting in elevated potassium content in composted material. In this study, biochar-amended vermicomposting resulted in elevated levels of potassium, reflecting the significant role played by biochar in retaining and making potassium enrichment during the process of decomposing press mud.

Carbon to nitrogen ratio signifies the maturity and stability of organic compounds during the decomposition process. It has been documented that C/N ratio decreases when various organic substrates are vermicomposted [12, 61, 66]. In the current study, the C/N ratio dropped in all the variants. The highest reduction was observed in C7 at 12.62, while the lowest was in C9 at 33.26. The lowering of C/N ratio might be the result of carbon loss during worm respiration and worm's gut microbial utilization. The observation of Bhattacharjee and Chaudhuri [67], Gupta and Garg [68], Gusain and Suthar [69] indicated that dropping of C/N ratio is due to increased nitrogen and phosphorous by microbial mineralization, furthermore the addition of their nitrogenous waste through excretion and mucus secretion. Wang et al. [55] found that biochar-amended composts had a more stable and lower C/N ratio as a result of increased microbial activity and nitrogen retention. During vermicomposting of press mud with biochar amendment, the C/N ratio gradually declined, suggesting improved nitrogen retention and faster organic matter decomposition.

The carbon-to-phosphorous ratio indicates that the compost substrate is decomposed during which the decline in TOC content and physical changes might have attributed to the increase of TP during the vermicomposting process. In the current study, the C/P ratio decreased significantly from initial day to final day in most of the combinations. But few maintained same percentage of 60th day in 75th day. The highest reduction was 19.34 in C7, while the lowest was 41.80 in C1. Balachandar et al. [20] have reported that the major reduction in total organic carbon, simultaneous increase in phosphorous contents and decomposition of substrate material during vermicomposting are the key processes which decrease the C/P ratio in the final product. Wang et al. [55] found that biochar-added compost had a lower C/P ratio compared to control treatments as a result of enhanced phosphorus mineralization and minimized leaching losses. In the current study, the inclusion of biochar led to a considerable decrease in the C/P ratio, suggesting increased phosphorus content and faster decomposition of organic matter in press mud vermicomposting.

The calcium (Ca) content showed a steady increase from the primary to the mature stage of vermicomposting. The highest increase in Ca level was 1.13 in C4, while the lowest was 0.85 in C11. The Na level reached a maximum of 0.41 in C4 and a minimum of 0.33 in C1 and C10. The Mg level was highest at 0.57 in C12 and lowest at 0.51 in C5 and C10. The S level peaked at 0.18 in C12, with the lowest value of 0.14 in C5 and C6. The rise in Calcium and magnesium may

Table 2 Initial (0th day) and final day (75th day) chemical-nutrient analysis (pH, EC, TOC, TKN, TP, TK, C/N ratio, C/P ratio) of pressmud mixed with cowdung amended with biochar

Treatments	Substrate	pH	EC (dS/m)	TOC (%)	TKN (%)	TP (%)	TK (%)	C/*N* ratio	C/*P* ratio
C1	Initial	7.96 ± 0.07	0.95 ± 0.01	41.55 ± 0.63	0.99 ± 0.02	0.72 ± 0.01	1.24 ± 0.01	42 ± 0.64	58.03 ± 0.58
	Final	7.77 ± 0.14	0.98 ± 0.09	32.56 ± 1.09	1.08 ± 0.06	0.78 ± 0.05	1.35 ± 0.07	30.26 ± 0.57	41.8 ± 1.37
% change (+/-)		-2.39^{ns}	3.16 ^{ns}	-21.64*	9.09*	8.33*	8.87*	-27.95*	-27.97*
C2	Initial	7.39 ± 0.04	1.09 ± 0.02	42.83 ± 0.76	1.07 ± 0.01	0.73 ± 0.02	1.24 ± 0.03	39.87 ± 0.91	59.03 ± 0.7
	Final	7.21 ± 0.07	1.12 ± 0.09	31.43 ± 0.76	1.2 ± 0.09	0.81 ± 0.07	1.39 ± 0.07	26.13 ± 0.69	38.68 ± 1.16
% change (+/-)		-2.44 ^{ns}	2.75 ^{ns}	-26.62*	12.15*	10.96*	12.10*	-34.46*	-34.47*
C3	Initial	7.6 ± 0.03	1.23 ± 0.01	45.2 ± 0.73	1.19 ± 0.02	0.73 ± 0.01	1.27 ± 0.02	38.07 ± 0.75	62.29 ± 0.88
	Final	7.41 ± 0.09	1.27 ± 0.06	27.97 ± 0.78	1.37 ± 0.11	0.84 ± 0.06	1.47 ± 0.1	20.44 ± 0.75	33.44 ± 0.93
% change (+/-)		-2.50 ^{ns}	3.25 ^{ns}	-38.12*	15.13*	15.07*	15.75*	-46.31*	-46.32*
C4	Initial	7.52 ± 0.05	1.22 ± 0.02	46.45 ± 0.93	1.14 ± 0.04	0.71 ± 0.01	1.3 ± 0.02	40.74 ± 0.6	65.73 ± 1.02
	Final	7.34 ± 0.1	1.25 ± 0.05	30.81 ± 0.91	1.24 ± 0.09	0.77 ± 0.06	1.41 ± 0.09	24.84 ± 0.85	40.08 ± 1.44
% change (+/-)		-2.39 ^{ns}	2.46 ^{ns}	-33.67*	8.77*	8.45*	8.46*	-39.03*	-39.02*
C5	Initial	7.76 ± 0.05	0.84 ± 0.01	44.89 ± 1	0.89 ± 0.04	0.78 ± 0.01	1.14 ± 0.02	50.67 ± 0.73	57.39 ± 0.97
	Final	7.42 ± 0.11	0.84 ± 0.04	28.77 ± 0.58	1.25 ± 0.1	1.07 ± 0.09	1.44 ± 0.09	23.05 ± 0.77	26.99 ± 0.98
% change (+/-)		-4.38 ^{ns}	0.00 ^{ns}	-35.91*	40.45*	37.18*	26.32*	-54.51*	-52.97*
C6	Initial	7.41 ± 0.03	0.98 ± 0.01	46.74 ± 0.69	0.88 ± 0.02	0.78 ± 0.01	1.17 ± 0.02	53.33 ± 1.01	59.76 ± 0.9
	Final	7.02 ± 0.12	1.03 ± 0.08	29.09 ± 0.97	1.8 ± 0.09	1.32 ± 0.1	1.78 ± 0.1	16.13 ± 0.76	22.09 ± 0.73
% change (+/-)		-5.26*	5.10*	-37.76*	104.55*	69.23*	52.14*	-69.75*	-63.04*
C7	Initial	7.38 ± 0.02	1.12 ± 0.01	48.78 ± 0.59	0.92 ± 0.02	0.8 ± 0.02	1.16 ± 0.03	52.83 ± 0.93	60.91 ± 0.75
	Final	6.81 ± 0.14	1.14 ± 0.1	33.08 ± 1.38	2.62 ± 0.14	1.71 ± 0.11	1.79 ± 0.1	12.62 ± 0.49	19.34 ± 0.54
% change (+/-)		-7.72*	1.79 ^{ns}	-32.19*	184.78*	113.75*	54.31*	-76.11*	-68.25*
C8	Initial	7.39 ± 0.04	1.28 ± 0.02	50.46 ± 0.73	1.09 ± 0.02	0.81 ± 0.01	1.19 ± 0.03	46.17 ± 0.63	62.27 ± 0.58
	Final	7.21 ± 0.09	1.32 ± 0.09	30.21 ± 0.87	1.7 ± 0.07	1.26 ± 0.08	1.69 ± 0.09	17.79 ± 0.66	23.95 ± 0.97
% change (+/-)		-2.44 ^{ns}	3.13 ^{ns}	-40.13*	55.96*	55.56*	42.02*	-61.47*	-61.54*
C9	Initial	8.09 ± 0.05	0.75 ± 0.01	47.21 ± 0.75	0.76 ± 0.01	0.8 ± 0.02	1.11 ± 0.02	61.86 ± 1.03	58.95 ± 0.78
	Final	7.93 ± 0.1	0.78 ± 0.06	36.57 ± 1.23	1.1 ± 0.06	1.14 ± 0.06	1.44 ± 0.06	33.26 ± 0.7	32.01 ± 0.79
% change (+/-)		-1.98 ^{ns}	4.00 ^{ns}	-22.54*	44.74*	42.50*	29.73*	-46.23*	-45.70*
C10	Initial	7.96 ± 0.06	0.8 ± 0.01	48.91 ± 0.81	0.8 ± 0.01	0.81 ± 0.01	1.14 ± 0.02	61.07 ± 0.78	60.36 ± 0.89
	Final	7.81 ± 0.13	0.82 ± 0.05	38.79 ± 0.91	1.22 ± 0.08	1.2 ± 0.09	1.54 ± 0.07	31.7 ± 0.94	32.26 ± 1.1
% change (+/-)		-1.88 ^{ns}	2.50 ^{ns}	-20.69*	52.50*	48.15*	35.09*	-48.09*	-46.55*
C11	Initial	7.82 ± 0.04	0.83 ± 0.01	49.58 ± 0.75	0.8 ± 0.01	0.83 ± 0.01	1.17 ± 0.02	61.9 ± 0.64	59.79 ± 0.91
	Final	7.66 ± 0.13	0.81 ± 0.05	39.64 ± 1.12	1.26 ± 0.05	1.24 ± 0.08	1.6 ± 0.08	31.42 ± 0.65	31.87 ± 1.26
% change (+/-)		-2.05 ^{ns}	-2.41 ^{ns}	-20.05*	57.50*	49.40*	36.75*	-49.24*	-46.70*
C12	Initial	7.44 ± 0.04	0.9 ± 0.01	50.78 ± 0.65	0.85 ± 0.02	0.82 ± 0.02	1.17 ± 0.03	59.87 ± 0.74	61.94 ± 0.78
	Final	7.3 ± 0.12	0.93 ± 0.06	42.59 ± 1.33	1.29 ± 0.07	1.19 ± 0.07	1.54 ± 0.07	33 ± 0.72	35.84 ± 1.27
% change (+/-)		-1.88 ^{ns}	3.33 ^{ns}	-16.13*	51.76*	45.12*	31.62*	-44.88*	-42.14*

The results are mean ±S.D. Values in % change with '+' and '-' signs indicate increase and decrease, respectively. '^{ns}' indicates not significant and '*' indicates significant by ANOVA ($P < 0.05$)

be due to the impact of organic acids formed in the process of decomposition that increases the mobilization of calcium and magnesium [70]. The increase in calcium, magnesium, and sulphur content in the biochar treatments implies that biochar directly impacts nutrient enrichment and retention during the composting process.

The growth and development of plants depend heavily on micronutrients, which are crucial nutrients for plants that are present in tissues in trace amounts. Plant nutrition would be impaired in the absence of these nutrients, possibly resulting in a plant productivity reduction. The Mn level was highest at 19.59 in C12 and lowest at 14.07 in C1. The Cu content ranged from a maximum of 19.74 in C12 to a minimum of 16.74 in C1. For Fe, the highest value was 119.41 in C12, while the lowest was 110.83 in C2. Zn levels peaked at 44.83 in C12 and were lowest at 38.80 in C2. In contrast, C11 exhibited a decline from the initial to the final day (Figs. 4 and 5). Higher levels of micronutrient in the vermicompost indicate that earthworms are gradually transformed the molecules of organic matter. The variables that lead to an improvement in micronutrients in the final product are bulking agent inclusion, biomasss volume reduction that concentrates the metal levels, and mineralization of organic waste [71, 72]. The addition of biochar further influences micronutrient levels by improving cation exchange capacity, enhancing microbial activity,

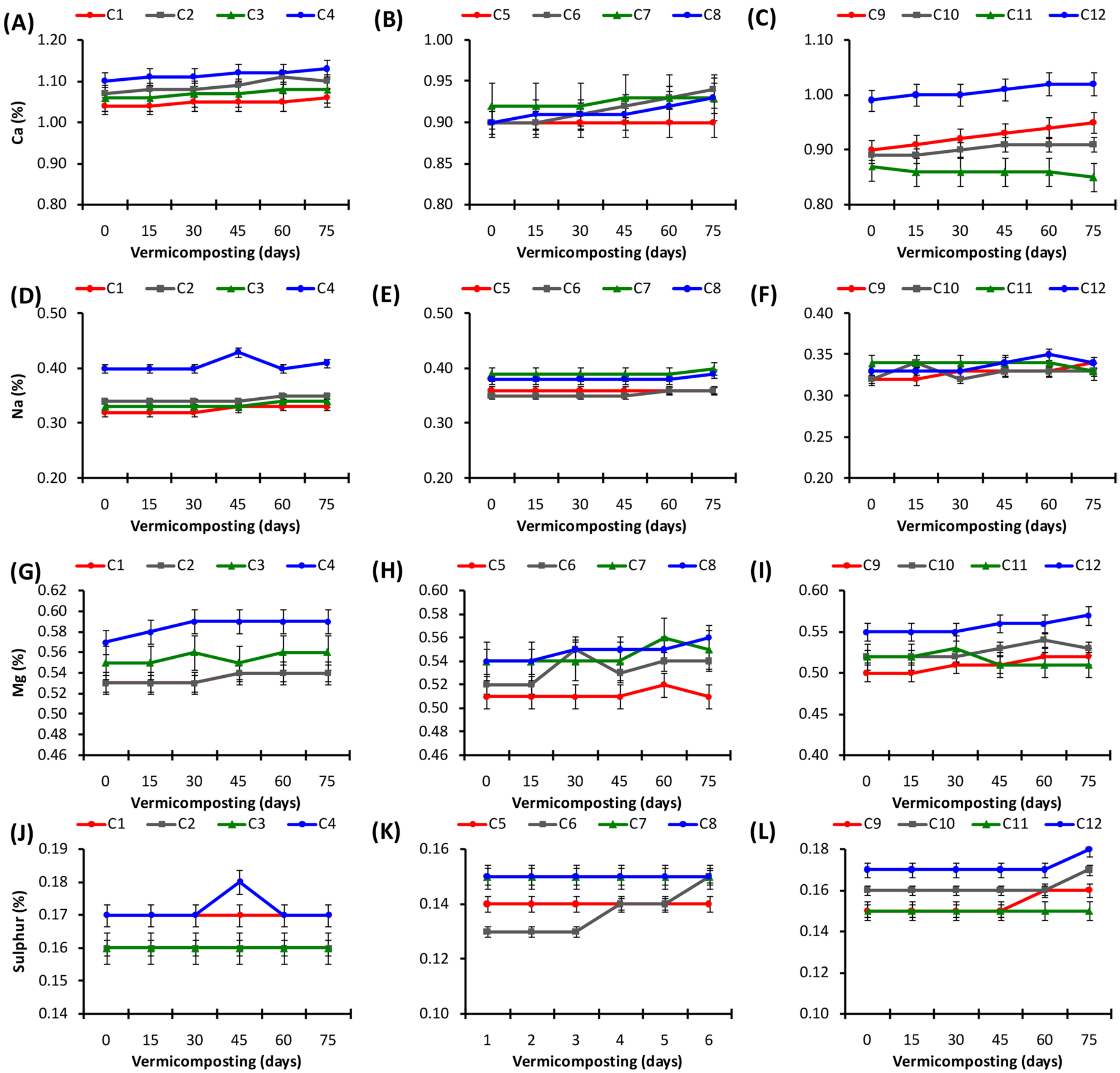

Fig. 4 The changes in Ca **A**–**C**, Na **D**–**F**, Mg **G**–**I**, and sulphur **J**–**L** contents during vermicomposting of pressmud + cow dung substrates amended with different proportions of biochar. Values are mean ± standard deviation (error bars)

and altering pH, which facilitates the content of essential micronutrients. However, excessive biochar application may lead to nutrient immobilization, negatively affecting overall compost quality [73, 74].

3.1 FTIR analysis of vermicompost

According to Bhat et al. [12], FTIR is a useful and trust worthy tool for identifying the functional group present in the raw materials and final product of vermicomposting. To understand the decomposition process, occur in vermicompost, it is necessary to assess the degree of mineralization and related biochemical changes. Analysis of the chemical structure of composting process provided by FTIR spectroscopy. It is a trustworthy tool for determining the compost maturity. Functional group act as an indicator band in the spectra which represent the components or metabolic products present and signal the stabilization or degradation process [12]. In this study, FTIR spectra of all vermicompost groups showed characteristic peaks between 3350 cm^{-1} and 1310 cm^{-1}, indicating the presence of aliphatic groups and lignin and other complex organic molecule breakdown.

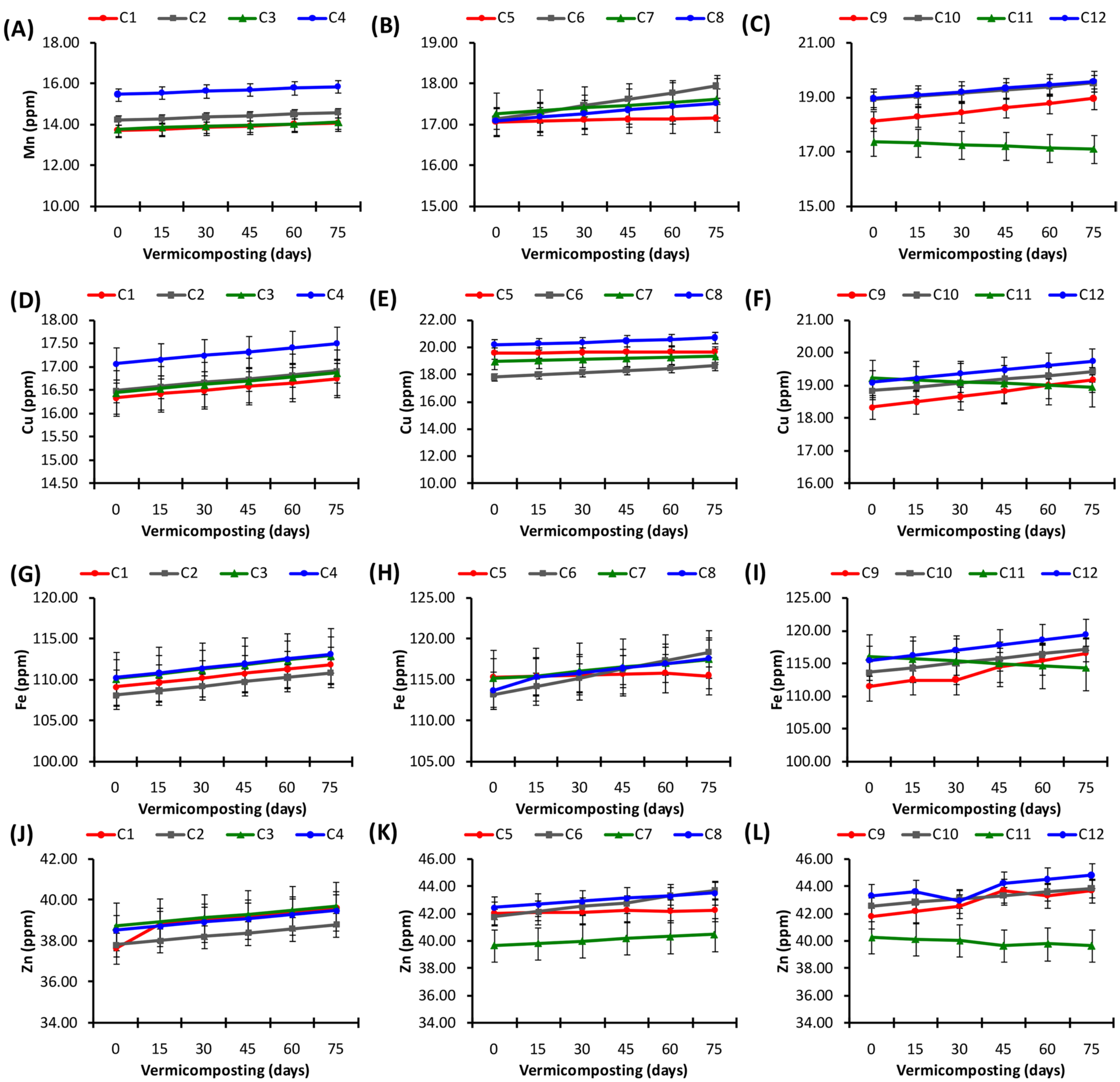

Fig. 5 The changes in Mn **A**–**C**, Cu **D**–**F**, Fe **G**–**I**, and Zn **J**–**L** contents during vermicomposting of pressmud + cow dung substrates amended with different proportions of biochar. Values are mean ± standard deviation (error bars)

Sen and Chandra [75] used FTIR spectra earlier to estimate humic acid content in sugar mill waste-derived compost, locating a broad band between 3400 cm^{-1} and 3300 cm^{-1}, corresponding to humic substances. This study concurs with their results, upholding that extensive degradation of the end vermicompost is indicated by the disappearance of carboxylic acid and aromatic structures. Specifically, the peaks of absorption between 3400 cm^{-1} and 2900 cm^{-1} point to the presence of carboxylic acid via O-H stretching. Absorption peaks within the range 2900 cm^{-1} to 1600 cm^{-1} indicate alkenes, while between 1400 cm^{-1} and 1200 cm^{-1} indicate alkanes via C-H bending. Moreover, amine groups are also detected between 1200 cm^{-1} and 1100 cm^{-1} via C-N stretching. The intensity of various bands changes during vermicomposting as lignin and complex carbohydrate like cellulose and hemicellulose break down [76, 77].

The end vermicompost had lower contents of polypeptides, polysaccharides, and aromatic compounds, indicating extensive mineralization and maturity of compost. Humification of organic matter has been proposed to be an increasing level of development in natural biodegradation and it is associated with the compost stability and maturity as well as their pathways to become highly humified [78, 79]. The enhanced presence of nitrogenous functional groups in the FTIR spectra also reflects the augmented degradation of biowaste, indicating efficient organic matter transformation. In addition, the addition of 4–6% biochar with pressmud and cow dung was observed to greatly enhance the nutrient content of the end vermicompost. This addition promoted decomposition of organic matter, resulting in higher humification and stabilization of the end product. These results highlight the function of biochar in enhancing microbial activity and maximizing the decomposition process (Fig. 6).

The multiple correlation analysis to compare the interaction of chemical nutrients present in the initial vermibed substrate (0 day) and final vermicompost (75th day) revealed that there exists between the major factors, pH, EC, TOC, TKN, TP, TK, C/N and C/P ratios while the other factors showed minor correlation (Table 3). The influence of pH on pH, EC, TOC, TKN, TP, TK, C/N ratio and C/P ratio vice-versa is well pronounced in the present study. The production of organic acids during the mineralization process jointly by earthworms and microbes formsa wide range of organic intermediates which in turn results in the change in the pH, EC and TOC mainly. Among the remaining parameters which are insignificant, the influence of sulphur was significant on Mn, Cu, and Fe.

3.2 SEM analysis

The Scanning Electron Microscopy (SEM) technique used in the present study served to offer meaningful information on changes in structure and texture during the vermicomposting process. The earlier studies by [80–83] utilized SEM to analyse the maturity of compost and vermicompost samples. SEM images from the present study showed that the earthworm *Eudrilus eugeniae* significantly differentiated and disintegrated in the final vermicompost in contrast to the initial vermicompost samples (Fig. 7). The SEM photographs of the current study demonstrated considerable variations in the first and last vermicompost samples, mainly caused by the biological activity of earthworm *Eudrilus eugeniae*. In the initial day of sample, the organic matter had a finely disintegrated but contiguous fibrous texture, indicating that the process of decomposition had commenced but was not established yet. Conversely, the final vermicompost exhibited an extremely fragmented, porous texture and more

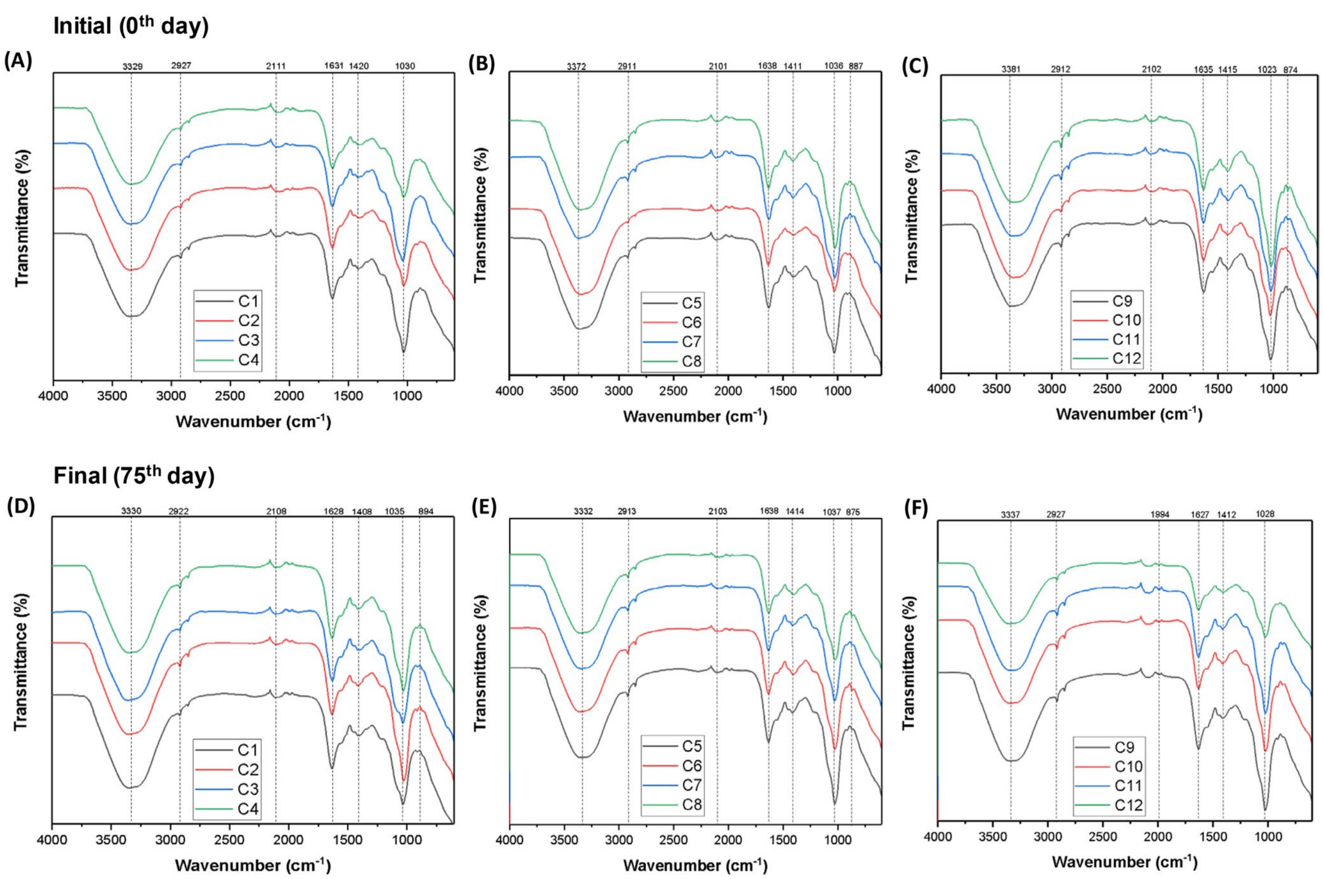

Fig. 6 FTIR spectra of biochar amended pressmud + cow dung combinations: initial **A**–**C** and final substrates **D**–**F** of different treatments C1-C12

Table 3 Multiple correlation analysis of nutrient changes during the vermicomposting of pressmud and cowdung combinations amended with biochar

Parameters	pH	EC	TOC	TKN	TP	TK	C/N	C/P	Ca	Na	Mg	S	Mn	Cu	Fe	Zn
pH	1															
EC	0.891	1														
TOC	0.753	0.916	1													
TKN	0.650	0.754	0.912	1												
TP	0.564	0.661	0.896	0.430	1											
TK	0.434	0.456	0.784	0.334	0.451	1										
C/N	0.650	0.750	0.990	0.892	0.239	0.340	1									
C/P	0.702	0.673	0.913	0.203	0.763	0.417	0.436	1								
Ca	0.135	0.587	0.231	0.107	0.219	0.289	0.394	0.272	1							
Na	0.135	0.201	0.117	0.088	0.090	0.140	0.311	0.330	0.271	1						
Mg	0.451	0.135	0.098	0.056	0.001	0.004	0.290	0.471	0.002	0.233	1					
S	0.337	0.334	0.105	0.216	0.020	0.022	0.204	0.003	0.049	0.036	0.355	1				
Mn	0.023	0.450	0.239	0.011	0.102	0.104	0.037	0.103	0.184	0.104	0.275	0.450	1			
Cu	0.219	0.257	0.113	0.126	0.003	0.233	0.293	0.150	0.210	0.320	0.090	0.506	0.102	1		
Fe	0.432	0.442	0.345	0.204	0.280	0.086	0.300	0.002	0.056	0.071	0.185	0.641	0.248	0.117	1	
Zn	0.136	0.875	0.012	0.137	0.004	0.005	0.121	0.029	0.114	0.008	0.005	0.347	0.190	0.094	0.081	1

Significant correlation (P=0.05) is indicated by grey shading in the table

surface irregularities, showing advanced decomposition and humification. This biological process resulted in a significant enhancement of porosity, which is one of the predominant characteristics of mature and stable vermicompost. The very high porous character of the produced final vermicompost not only supports water retention and oxygenation but also encourages healthy microbial activity, further adding to the quality of the compost. The findings of SEM analysis of this study support the findings of the previous research of [81, 83, 84]. The enzymes which are responsible for the degradation of organic materials are secreted by the gut associated microbes who found in earthworm intestine [12]. When compared to the initial vermicompost samples, the morphology of the final vermicompost in the present study indicated maximum increase of decomposition. (Fig. 7).

4 Summary

This work investigated vermicomposting with biochar as a sustainable method for the utilization of sugarcane press mud. A press mud-cow dung mixture containing *Eudrilus eugeniae* and biochar (2%, 4%, 6%) was used by the study to augment decomposition, nutrient conservation, and compost quality. The process was followed by 30-day precomposting, after which 75-day vermicomposting was carried out and tested via SEM, FTIR, andchemical analysis. Evidences proved that earthworms enhanced degradation, biochar enhanced microbial processes, and their combination improved stability of the compost. The process is an environmentally friendly method for recycling organic waste and organic farming, providing a substitute for chemical fertilizers.

5 Implication

This research emphasizes the sustainable management of sugarcane waste through the incorporation of biochar in vermicomposting. The method increases soil fertility, enhances nutrient retention, and speeds up decomposition with the aid of *Eudrilus eugeniae*. It decreases the use of chemical fertilizers, facilitates recycling of waste, and provides an affordable option for organic farming. Moreover, biochar-amended vermicompost is commercially viable and can be modified for global agro-industrial waste management, enabling a circular economy and environmental sustainability. The work is confined to controlled conditions and which may differ from field conditions. Long-term soil and plant impact requires further investigation, and feasibility at large scale should be examined for practical utilization.

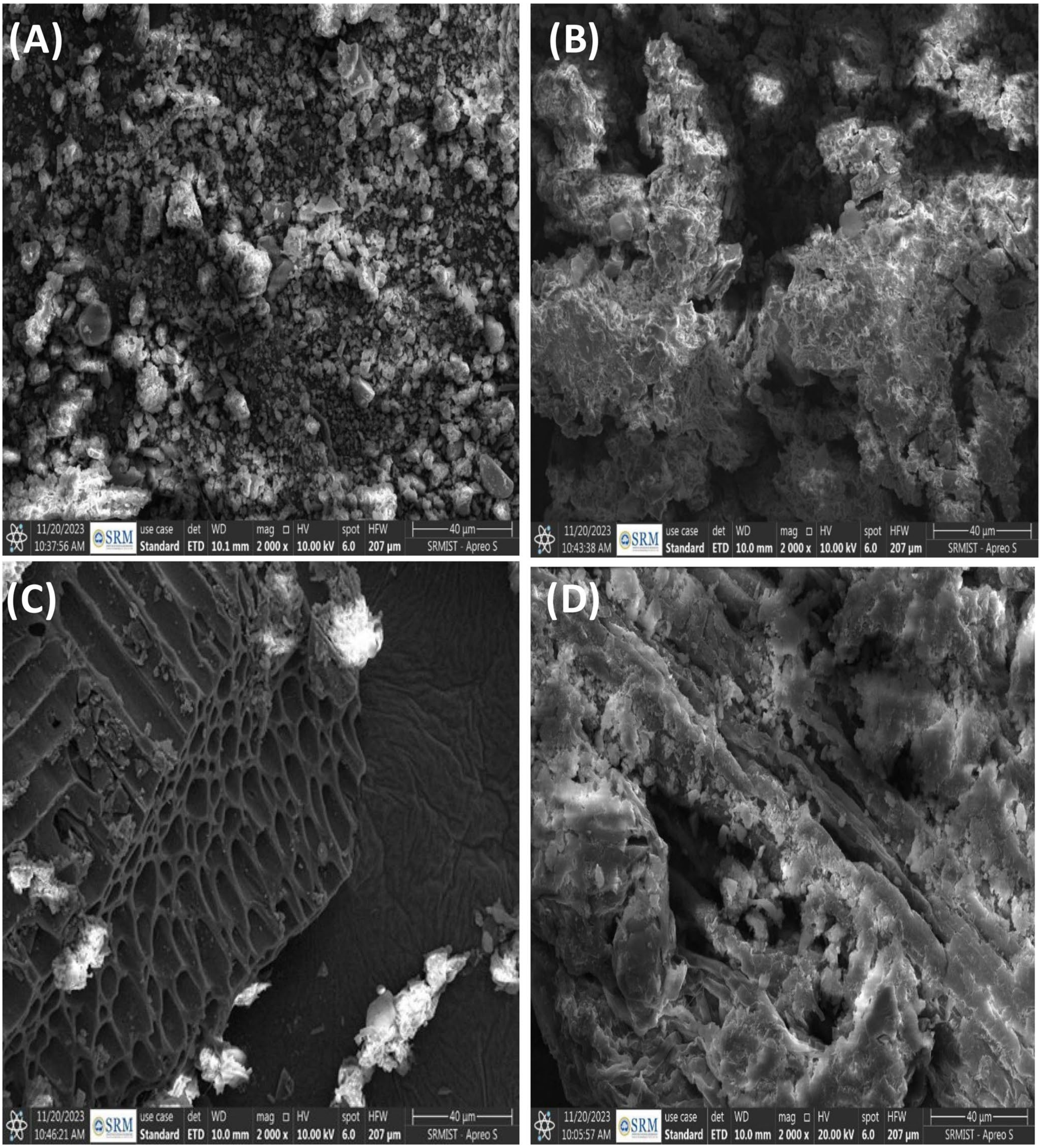

Fig. 7 SEM result of the pressmud and cow dung mixtures amended with biochar. **A** initial C3 substrate, **B** final C3 vermicompost, **C** initial C7 substrate, **D** final C7 vermicompost

6 Conclusion

The current research showed that vermicomposting is a viable and eco-friendly approach for the utilization of sugar mill waste, specifically pressmud, as a nutrient-enriched organic manure. Utilization of *Eudrilus eugeniae* greatly promoted the process of decomposition, which resulted in high-quality vermicompost production. The incorporation of biochar as an additive amendment further increased the efficacy of vermicomposting by regulating aeration, microbial activity, and heavy metal sequestration. Between the various biochar treatments, 4% and 6% biochar were the most efficient in promoting nutrient availability and compost quality. The chemical, SEM, and FTIR characterization validated the enhanced stability, structure, and nutritional profile of the vermicompost as a promising substitute to chemical fertilizers. In general, this research underscores the future prospect of biochar-amended vermicomposting as a green solution for the sustainable management of sugarcane waste, and enhancement of soil fertility for sustainable agriculture.

Author contributions Vasantha Karunakaran Rekha: Conceptualization, Methodology, Investigation, Formal analysis, Writing - original draft; Subbiah Manivannan: Conceptualization, Methodology, Investigation, Formal analysis, Writing - original draft; Gopalakrishnan Abirami: Methodology, Investigation, Formal analysis, Writing - original draft, Writing - review & editing; Malaiyandi Jayanthi: Methodology, Software, Resources, Investigation, Formal analysis, Writing - review & editing; Muthusamy Suganthi: Investigation, Methodology, Resources, Formal analysis, Writing - review & editing; Ramachandran Ananthavalli: Methodology, Resources, Software, Formal analysis, Writing - review & editing; Krishna Kumar Ashok Kumar: Conceptualization, Methodology, Resources, Formal analysis, Writing - review & editing, Supervision.

Data availability The datasets used can be accessed as requested by the authors.

Declarations

Ethical approval Not applicable.

Competing interests The authors declare no competing interests.

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