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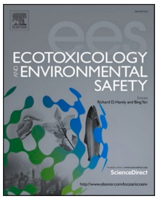

Feasibility of vermicomposting for spent drilling fluid from a nature-gas industry employing earthworms *Eisenia fetida*

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ABSTRACT

This study investigated the vermicomposting of spent drilling fluid (SDF) from the nature-gas industry mixed with cow dung in 0% (T1), 20% (T2), 30% (T3), 40% (T4), 50% (T5), and 60% (T6) ratio employing *Eisenia fetida* under a 6 weeks trial. *Eisenia. fetida* showed better growth and reproduction performances in the first three vermireactors (T1–T3), and the mortality was higher in the vermireactors that contained more spent drilling fluid ($\geq$ 40%). Vermicomposting results in a decrease in total organic carbon, C/N ratio, and an increase in EC, total nitrogen, total phosphorous, total potassium compared to their initial values. The RadViz and VizRank showed that vermicomposting results in a greater impact on the C/N ratio (15.24–35.48%) and EC (7.29–26.45%) compared to other parameters. Activities of urease and alkaline phosphatase during vermicomposting initially increased and then declined suggesting vermicompost maturity. Also, seed germination, mitotic index and chromosomal abnormality assays using cowpea signified that the vermicomposts T2 is suitable for agricultural use due to the lower phytotoxicity and cytotoxicity. The results indicated that SDF could be converted into good quality manure by vermicomposting if mixed up to 20% with cow dung.

1. Introduction

Drilling fluids are used during exploratory and development drilling for oil and natural gas to lubricate and cool the drilled bit and control subsurface wellbore pressure (Yao and Naeth, 2014). Upon completion of a well, an increased volume of spent drilling fluid (SDF) results from the disintegration of rock cuttings transport to the surface. During 2016–2019, approximately 18,000 and 7000 wells were drilled for crude oil and nature gas just in Changqing oilfield, which is the largest oil and gas supplier in China. The volume of the drilling waste generated during construction and drilling is nearly 300–800 m^3 per well. Thus, an estimated 1,300,000 m^3 of drilling waste in this region will be disposed of. Many hundreds of thousands occur throughout the world, thus posing a serious environmental issue to be addressed.

Many methods have been applied in SDF management for decades, such as chemical stabilization and solidification, used in construction, transport to an approved landfill, and land reclamation (Saintfort and Ashtani, 2014; Mostavi et al., 2015). Previous studies have revealed that SDF can be utilized as a soil amendment to improve the water-retaining capacity and hydraulic conductivity (Mikosszymanska et al., 2018). The high content of macro- (e.g., phosphorous and potassium) and micro-nutrients (e.g., ion, zinc, and manganese) in SDF are essential for plant growth (Bauder et al., 2005; Yao and Naeth, 2014). Despite studies revealed that the SDF comprises underutilized resources that can be recycled for soil fertility improvement in either agriculture or the remediation of degraded lands (Molina et al., 2013; Wang et al., 2014). Application of SDF directly to the soil before stabilization is not recommended since immature components might affect plant growth

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Abbreviations: ALP, alkaline phosphatase; CD, cow dung; EC, electrical conductivity; RENR, relative efficiency of nutrient recovery; SDF, spent drilling fluid; TOC, total organic carbon; TKN, total Kjeldahl nitrogen; TK, total potassium; TP, total phosphorus; NH_4^+, ammonium; NO_3^-, nitrate; RadViz, Radial Coordinate Visualization; UA, urease.

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owing to nitrogen deprivation, generation of toxic metabolites, too much input of metals or inorganic salts (Wang et al., 2016; Malinska et al., 2017). Moreover, considerable amounts of organic and inorganic nutrients that locked in organic wastes are reduced or lost in the form of greenhouse gases (Meng et al., 2017). Hence, environmentally friendly and economically feasible technologies for waste stabilization before direct land application are a high priority.

Vermicomposting is an efficient way to stabilize organic waste due to its easy adoption, faster degradation, enhanced macro-and micro-nutrients, beneficial microorganisms along with plant growth-promoting substances (Singh et al., 2020). There is evidence of successful utilization of vermicomposting for stabilizing different organic waste such as animal manure, plant waste, and industrial sludge (Romero et al., 2007; Lv et al., 2016). Based on the ecological niche, the epigeic category of earthworms is bested suited for the vermicomposting technology due to their voracious feeding habit, wider acceptability of organic waste materials, and high fecundity (Rini et al., 2020). In this respect, *Eisenia fetida* as the most dependable epigeic species has been employed for organic waste management throughout the world (Bhattacharya and Kim, 2016; Singh et al., 2020). For more efficient vermicomposting, an amendment material is very important as it serves as the initial nutrient material for earthworms and microbial proliferation. Cow dungs (CD) as bucking material enhanced the palatability of biowaste substrates, enhances the mineralization process, lowers the C/N ratio to desirable levels for microbial and earthworm action (Devi and Khwairakpam, 2020). Researchers have used cow dungs as the sole vermicomposting substrates or in combination as a bulking agent with organic solid waste to convert them into more stabilized nutrient-rich vermicompost for studying the growth and reproduction of earthworms (Devi and Khwairakpam, 2020; Paul et al., 2020; Rini et al., 2020; Karmegam et al., 2021). Also, the agronomic qualities of the final product essentially require the amendment of cow dungs during vermicomposting.

To the best of the authors' knowledge, no comprehensive study on the vermicomposting of spent drilling fluid is yet conducted by any research group. Therefore, the objective of the current study is to investigate the possible utilization of SDF from the natural gas field of Changqing oilfield in vermicomposting by employing the earthworm species *Eisenia fetida*. To accomplish this, the study focused on the following aspects: (I) feasibility of SDF vermicomposting through the assessment of earthworm population dynamics (survival, growth, and reproduction); (II) the quality of final products through the analysis of key agronomical parameters such as NPK nutrients content and maturity/stabilization indicators and, (III) assess the potential toxicity of the final products through the phytotoxic and genotoxic parameters such as germination index, mitotic index, and chromosomal abnormality.

2. Methods and materials

2.1. Sample collection

Fresh samples of the SDF (water-based) used in this study were obtained from active drilling sites situated at Changqing Oilfield (Shaanxi, China). Spent drilling fluid with ~75% moisture content was collected in a round-shaped plastic container, and excess black-colored wastewater was removed and dried at room temperature. The CD for this study was purchased in fermented form from a nearby cattle shed and used for further experiments. The main physicochemical characteristics of the SDF and CD are given in Table 1. Healthy worms (*Eisenia fetida*) were randomly inoculated in the laboratory with CD as a culture medium to adapt to the laboratory conditions before the experiment began.

2.2. Experimental designing and vermicomposting

The vermicomposting experiments were carried out in the laboratory using plastic containers as reactors (30 cm × 15 cm × 18 cm, length × width × depth). A total of seven feed mixtures (dry weight basis) of SDF and CD were used for the experiment, as presented in Table 2. Experimental beddings were prepared by placing pieces of small stones and leaf litter at the bottom of each reactor (Suthar and Singh, 2008). Day 0 refers to the day of inoculation of earthworms after pre-composting of 1 week to eradicate volatile toxic gases (if any) and to make the feed mixtures palatable to worms (Elvira et al., 1996). For vermicomposting, sixty healthy and non-clitellated (no saddle-shaped thickening of the body wall) earthworms (live weight 271–290 mg) were introduced into different reactors containing 500 g of the initial feed mixtures. All vermireactors were covered by the plastic film and keep in darkness at room temperature (~25 °C) for 6 weeks. The moisture level was maintained at ~65% by sprinkling distilled water onto the compost medium when necessary. During the study period, no extra feed was added at any stage. Homogenized samples of all feed mixtures were drawn at 0 days (initial) and 6 weeks (final). All samples were divided into two subsamples, one of which was air-dried at room temperature and finely ground for chemical analysis, while the other was stored at − 20 °C for enzyme activities analysis.

Table 1
Physic-chemical characteristics of the raw material used in this experiment.

S.no.	Parameters	SDF	CD
1	pH (unitless)	9.93	8.03
2	EC (dS m^{-1})	7.66	2.77
3	TOC (g kg^{-1})	118.26	441.97
4	TN (g kg^{-1})	1.96	16.24
5	TP (g kg^{-1})	0.33	3.62
6	TK (g kg^{-1})	16.59	5.58
7	C/N (unitless)	62.15	27.24
8	C/P (unitless)	202.45	112.88

EC, electrical conductivity; TN, total nitrogen; TOC, total organic carbon; TK, total potassium; TP, total phosphorus; C/N, carbon to nitrogen ratio.

2.3. Physicochemical analysis

The air-dried samples were used for the analysis of physicochemical parameters. All the samples were analyzed in triplicate and the average results were used for comparisons. The pH and electrical conductivity (EC) were determined using a ddH_2O suspension of each vermicompost in the ratio of 1:10 (wt/vol). Total organic carbon (TOC) was determined after igniting the sample in a Muffle furnace at 550 °C for 1 h. The total Kjeldhal Nitrogen (TKN) was determined by using the Kjeldhal method. Nitrate (NO_3^-) and ammonium (NH_4^+) were measured by a Lachat Flow Analyzer (AutoAnalyzers3-AA3) after extracting samples using 1 M KCl. Total phosphorus (TP) was analyzed using the spectrophotometric methods with molybdenum in sulfuric acid and total potassium (TK) was determined by flame photometer. The ratio of the percentage of carbon to that nitrogen (C/N) was calculated by dividing the estimated percentage of carbon by the percentage of nitrogen for each sample. The humification index (HI) was determined by employing the method outlined by Zbytniewski and Buszewski (2005). The relative efficiency

Table 2
Composition of treatments used for experimentation.

Treatment	Treatment description	SDF (g)[a]	CD (g)[a]	Earthworm
T1	CD (100%)[b]	–	500	60
T2	SDF (20%) + CD (80%)	100	400	60
T3	SDF (30%) + CD (70%)	150	350	60
T4	SDF (40%) + CD (60%)	200	300	60
T5	SDF (50%) + CD (50%)	250	250	60
T6	SDF (60%) + CD (40%)	300	200	60
T7	SDF (100%)	500	–	60

SDF - spent drilling fluid; CD - cow dung.

[a] Dry weight basis.

[b] The figure in parentheses indicates the percent in the initial substrate material.

of nutrient recovery (RENR) for TKN, TP, and TK of vermicomposts with SDF compared to the control (without SDF in the feed mixture) was calculated according to the methods adopted by Swarnam et al. (2016).

2.4. Activities of enzyme analysis

Samples from all the treatments were collected on 0 (initial), 2, 4, and 6th week of experimentation and used for the analysis of the enzymes, urease (UA), and alkaline phosphatase (ALP). The activity of UA was assessed by the rate of release of NH_4^+ from the hydrolysis of urea as previously described (Feyzi et al., 2020). The activity of soil extracellular ALP was estimated by measuring the release of p-nitrophenol (PNP) from p-nitrophenyl phosphate (PNPP) (Karmegam et al., 2019).

2.5. Growth and reproduction of earthworms

To study the growth and reproduction of earthworms, the cow dung (CD) was used as a standard background medium that served as a control. Earthworms, hatchlings, and cocoons were separated from the substrate material by manual sorting, after which the worms were counted and weighted after washing with water. Growth parameters of the earthworms, i.e., biomass, cocoon production rate, mortality, and fecundity rate, were calculated as previously described by Suthar (Suthar, 2009a).

2.6. Germination test and genotoxicity assay

The vermicompost maturity was assessed by seed germination studies with cowpea (*Vigna unguiculata* L.) using vermicompost extracts obtained from each treatment. For this study, extracts of fresh vermicompost were prepared with distilled water at a ratio of 1:10 (wt/vol) according to previous methods, and treatment with double-distilled water (DW) served as a control (Karmegam et al., 2019). Seeds were sown in covered Petri dishes (20 seeds per dish, five replicates) to avoid the solvent evaporation, on two filter paper layers imbibed with 10 ml of extracts and incubated in a germination chamber. Seed germination was scored daily for 4 days, and the germination percentage (GP) was calculated by dividing the number of germinated seeds by the total number of tested seeds for each treatment. The germination index (GI) was calculated with the double-distilled water as the control employ the methods described by Khatua et al. (2018).

Genotoxicity assays were also conducted using extracts of fresh vermicompost and included treatment with DW. Root apices from 4-day-old seedlings were excised, and immediately fixed in Formalin/acetic acid/alcohol (FAA) for 24 h, and then preserved in 70% ethanol for further analysis. The fixed roots were treated with 1 mol/L HCl at 60 °C for 15 min. The genotoxicity effect was evaluated by calculating the mitotic index (MI) and chromosomal aberrations (CA) observed in meristematic cells following methods described previously (Pandey, 2008). Five slides were prepared per treatment to evaluate the MI and CA, and six fields per slide were evaluated. Micronucleus (MN) frequency was also counted in meristematic cells. The chromosomal aberrations were detected with micrographs using an Olympus BX51 at a total magnification of 40 × 10. The most frequent abnormalities are shown in photomicrographs.

2.7. Statistical analysis

The differences in physicochemical characteristics between different reactors were statistically interpreted using one-way analysis of variance (ANOVA). Tukey's *t*-test was performed to identify homogeneous reactors in terms of the differences in chemical parameters between the initial feed mixtures and the final vermicompost products. The statistical analysis was carried out in JMP 10.0 software. Radial coordinate visualization (RadViz) was used to analyze the underlying variation between the initial feed mixtures and the final vermicompost; all chemical parameters were employed to obtain a clear and good separation using only 5 parameters. VizRank was used to score the visualization projections according to the degree of class separation and possible projection candidates were investigated to find the highest scores (Leban et al., 2005). In this article, the *k*- nearest neighbor (*k*-NN) was implemented in VizRank to score a specific projection, and then the score of the projection was estimated through a leave-one-out process where each data point was classified using *k*-NN classifier obtained from all other data instances in the projected space. The single first-ranked projection was used to present the classification for the different data sets. VizRank and RadViz analyses were carried out in Python 3.7 (www.python.org).

3. Results and discussion

3.1. Earthworm growth and reproduction performance

The growth and survival of earthworms used for vermiconversion of organic waste are considered good indicators of an effective vermicomposting process (Karmegam et al., 2019). In the current study, the earthworms did not accept 100% SDF as a feed and almost no surviving earthworms were found at the end of this experiment. The addition of CD to SDF improved the environmental conditions for the growth and survival of earthworms. The changes in worm biomass for all feed mixtures at the end of experiments are presented in Table 3. There was a significant difference (ANOVA, $P < 0.05$) among all vermireactors for the individual live weight and individual weight gain biomass in worms ($F = 547.161$ and 590.322, respectively). The maximum individual live weight was observed in vermireactor T4 (434.21 mg earthworm^{-1}) followed by T3, T2, T1, T5, and T6. The maximum individual weight gain biomass was observed in vermireactor T4 (148.21 mg earthworm^{-1}), followed by T3 (148.03 mg earthworm^{-1}). A statistically significant difference was observed between vermireactors T4 and T1 (100% SDF) (Tukey's *t*-test; $P = 0.007$). However, a dramatic decrease in individual weight gain biomass was observed in vermireactors T5 and T6 (16.05 and − 145.84 mg earthworm^{-1}). In general, earthworm biomass gain is directly dependent upon the feeding rate, the quantity and nature of the ingestible substrate, and the microbial richness in feed mixtures (Neuhauser et al., 1980; Gomez-Brandon et al., 2011). For the total biomass of earthworms at the end of the experiment, there was no difference among any vermireactors except T5 and T6 (Fig. 1A). Most likely, the poor nutritional quantity of SDF, such as low contents of nitrogen and easily digestible components, had adverse effects on the growth habits of earthworms in feed mixtures containing higher contents of SDF. Earthworm mortality earthworms showed a significant difference (ANOVA, $F = 35.969$, $P < 0.05$; Fig. 1B) among all vermireactors, and a positive correlation was observed in all vermireactors with increasing proportions of SDF in the feed mixtures. The number of surviving earthworms was ranged from 31.33 to 55.67 at the end of the experiment (Table 3). The maximum mortality was found in vermireactor T6 (47.78%) followed by T5 (26.67%), T4 (15.56%), T3 (11.66%), T1 (11.67%), and T2 (7.22%). There is no statistical difference between T1, T2, and T3 ($P < 0.05$). The reason could be interpreted as the nonpalatability and toxicity of SDF. The EC and pH of feed mixtures are limiting factors for the survival and growth of earthworms. Mitchell suggested that for earthworms, the maximum tolerance limit for pH is 9.5, and that of EC is 5.0 dS m^{-1}. In the present study, the higher pH and EC values exceeding the tolerance limits of earthworms in the feed mixtures containing more SDF (T5 and T6), caused higher mortality at the end of the experiment. The minimum mortality of earthworms was detected in T2 (7.22%), and there was no significant difference between T1 and T2 (Tukey's *t*-test; $P = 0.208$), indicating that low proportions of SDF in the feed mixture did not impact the survival of earthworms. Therefore, the CD can act as a complementary waste to convert SDF into quality manure.

The reproduction of earthworms was also recorded at the end of the

Table 3

Growth and reproduction of earthworms in different feed mixtures after 6 weeks of vermicompost (Mean ± S.D., n = 3); mean values followed by different letters are statistically different among all vermireactors (ANOVA, Tukey-HSD., $P < 0.05$).

Treatment	Number of surviving earthworms	Initial individual live weight (mg)	Individual biomass gain (mg)	Final individual live weight (mg)	Cocoons produced in the experiment
T1	53.00 ± 1.63	271.67 ± 6.34	100.71 ± 12.62b	372.38 ± 12.61b	272.67 ± 4.64a
T2	55.67 ± 1.25	272.67 ± 7.41	112.36 ± 6.18b	385.04 ± 6.18b	258.00 ± 8.61a
T3	53.67 ± 1.25	281.33 ± 7.58	148.03 ± 3.76a	429.36 ± 3.76a	99.67 ± 5.73b
T4	48.67 ± 1.69	286.00 ± 4.08	148.21 ± 3.34a	434.21 ± 3.34a	19.66 ± 3.29c
T5	44.00 ± 2.16	286.67 ± 4.49	16.05 ± 3.36c	303.94 ± 3.26c	12.00 ± 2.45c
T6	31.33 ± 3.39	290.33 ± 4.63	−145.84 ± 5.17d	144.48 ± 5.17d	ns
Treatment	Reproduction rate (cocoons worm^{-1})	Juveniles produced during experimentation	Total population at the end	Facundity rate (juveniles worm^{-1})	
T1	5.15 ± 0.23a	894.00 ± 34.56a	947.00 ± 36.20a	16.89 ± 0.95a	
T2	4.63 ± 0.24b	298.00 ± 25.94b	353.67 ± 25.94b	5.35 ± 0.41b	
T3	1.88 ± 0.08c	88.33 ± 10.44c	141.33 ± 12.07c	1.66 ± 0.14c	
T4	0.38 ± 0.06d	16.67 ± 5.44d	67.33 ± 3.29d	0.32 ± 0.04d	
T5	0.27 ± 0.04d	10.00 ± 3.26d	54.00 ± 5.35d	0.22 ± 0.06d	
T6	ns	ns	31.33 ± 3.39e	ns	

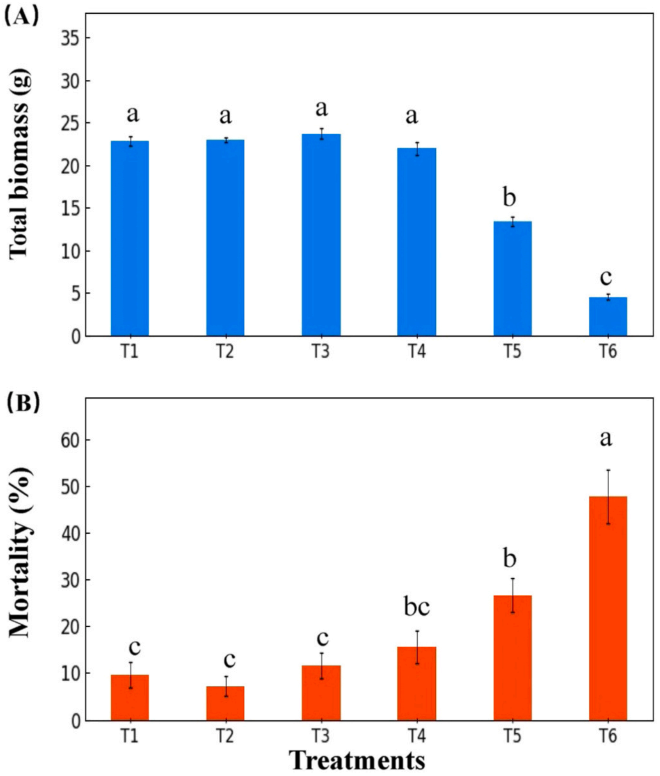

Fig. 1. Total biomass (A) and mortality (B) of earthworms at different vermireactors at the end experiment (Mean ± S.D., n = 3). Different lowercase letters in each column represent significant differences among all treatments, at the $P < 0.05$ level, based on the Tukey-HSD.

experiment (Table 3). The earthworms showed significantly differences (ANOVA, $P < 0.05$) in total cocoon number (F = 1283.328), mean production rate (cocoons worm^{-1}) (F = 457.127), juvenile number (F = 931.368), fecundity rate (F = 469.151) and final total population (F = 950.544) among the different vermireactors. A significantly higher mean production rate was observed in vermireactor T1 (5.15 cocoons worm^{-1}) followed by T2 (4.63), T3 (1.88), T4 (0.38), and T5 (0.27) during vermicomposting process ($P < 0.05$, for all). The maximum cocoon number was detected in vermireactor T1 (272.67), and there was no significant difference between T2 and T1 (Tukey's *t*-test; $P = 0.101$). The hatchling number directly depends upon the cocoon hatching success and the survival rate of newly emerged worms in feed mixtures (Negi and Suthar, 2013). The results showed that the variation in hatchling numbers among different vermireactors consisted of the cocoon production. The maximum hatchling number was produced in vermireactor T1 (894.00) followed by T2 (298.00), T3 (88.33), T4 (16.67), and T5 (10.00). The data revealed that the addition of up to 30% SDF in the initial feed mixture did not support the cocoon production and that the earthworms could not successfully reproduce. In addition, ammonia, nitrogen oxide, carbon dioxide, and other intermediate products during vermicomposting may impact the survival of newly emerged worms. The total population of earthworms after 6 weeks vermicomposting showed significant differences (ANOVA, F = 950.544, $P < 0.05$) among different vermireactors and were negatively correlated with increasing SDF concentration in the feed mixtures. A significantly higher total population was observed in vermireactors T1 (947.00) followed by T2 (235.67), T3 (141.33), T4 (67.33), T5 (54.00), and T6 (31.33) ($P < 0.05$, for all).

3.2. Vermicompost quality

Vermicomposting with earthworms can convert some organic matter into worm biomass and the rest is converted into nutritious vermicompost. In this process, an appropriate feed composition is very important for the worms to optimize the manual value of the vermicompost. To assess the nutrient quality of the vermicompost product and the suitability of the feed mixtures for vermiconversion, physicochemical analysis of the initial feed mixtures (with different contents of CD and SDF) and the final vermicompost product (after vermicomposting for 6 weeks) was performed, and the results are presented in Table 4 and Fig. 2.

3.2.1. Changes in pH and electrical conductivity (EC)

Vermicomposting increased the pH values in the final vermicompost compared to their initial levels (Table 4). The initial pH was in the range of 8.02–9.55 and that changed to 8.37–9.67 at the end of vermicomposting. There was about 1.68% (T6) to 4.07% (T3) increment in pH after the vermicomposting process and the variation was significant among different vermireactors (ANOVA, F = 5.641, $P < 0.05$). The change of pH during the vermicomposting could be due to the difference in the activity of earthworms and microorganisms. It has been reported that the increased pH in the feed mixture during the initial phase of vermicomposting and declined at the later phase of the vermicomposting (Paul et al., 2020; Saez et al., 2021). The increase of pH could be attributed to organic N (especially protein degradation) mineralization during the bioconversion process. Alkali-C and organic salts, formed during the organic matter decomposing also contributed to pH increment in the vermicompost (Rai and Suthar, 2020). The results revealed that there was no significant difference in pH between the final products and initial feed mixtures (Tukey's *t*-test, $P = 0.113$, Table 4). This can be attributed to the neutralizing effects of earthworm activity such as the generation of phenolic compounds and organic acids, and also due to the

Table 4
Variations of pH, EC, C/N, and C/P ratio in different feed mixtures after 6 weeks of vermicompost (Mean ± S.D., n = 3).

Treatments	pH			EC (ds/m)		
	Initial	Final	% increase over initial	Initial	Final	% increase over initial*
T1	8.02 ± 0.05	8.37 ± 0.06	3.94a	2.82 ± 0.06	3.65 ± 0.06	26.45a
T2	8.38 ± 0.05	8.71 ± 0.04	3.41ab	3.25 ± 0.04	3.81 ± 0.10	18.72b
T3	8.78 ± 0.03	9.15 ± 0.06	4.07a	3.47 ± 0.03	4.04 ± 0.05	16.64b
T4	9.08 ± 0.03	9.39 ± 0.05	3.16ab	3.84 ± 0.05	4.23 ± 0.13	10.15c
T5	9.35 ± 0.05	9.59 ± 0.04	2.53ab	4.26 ± 0.09	4.46 ± 0.16	7.29c
T6	9.55 ± 0.03	9.67 ± 0.04	1.68b	4.79 ± 0.07	5.19 ± 0.04	8.43c
Treatments	C/N ratio			NH_4^+-N/NO_3^--N		
	Initial	Final	% decrease over initial*	Initial	Final	% decrease over initial*
T1	27.24 ± 0.67	17.56 ± 0.17	35.49a	15.92 ± 1.47	4.79 ± 0.21	69.29c
T2	29.49 ± 0.64	23.88 ± 0.31	17.28bce	26.27 ± 2.99	0.79 ± 0.04	96.96a
T3	30.27 ± 1.87	23.55 ± 0.82	20.81bce	16.98 ± 1.74	1.15 ± 0.02	93.21a
T4	30.32 ± 1.79	24.72 ± 0.57	18.24bce	14.35 ± 2.06	1.92 ± 0.22	85.96b
T5	35.58 ± 2.35	28.65 ± 0.97	21.08b	6.67 ± 0.61	1.78 ± 0.05	72.94c
T6	38.50 ± 2.15	32.49 ± 0.78	15.24c	8.09 ± 0.26	8.94 ± 0.94	7.25d

* - % decrease/increase values between the initial and final vermicompost are significantly different at $P < 0.05$ by paired *t*-test; Different lowercase letters in % decrease/increase values in initial and final vermicompost between treatments represent significant differences at the $P < 0.05$ level based on the Tukey-HSD.

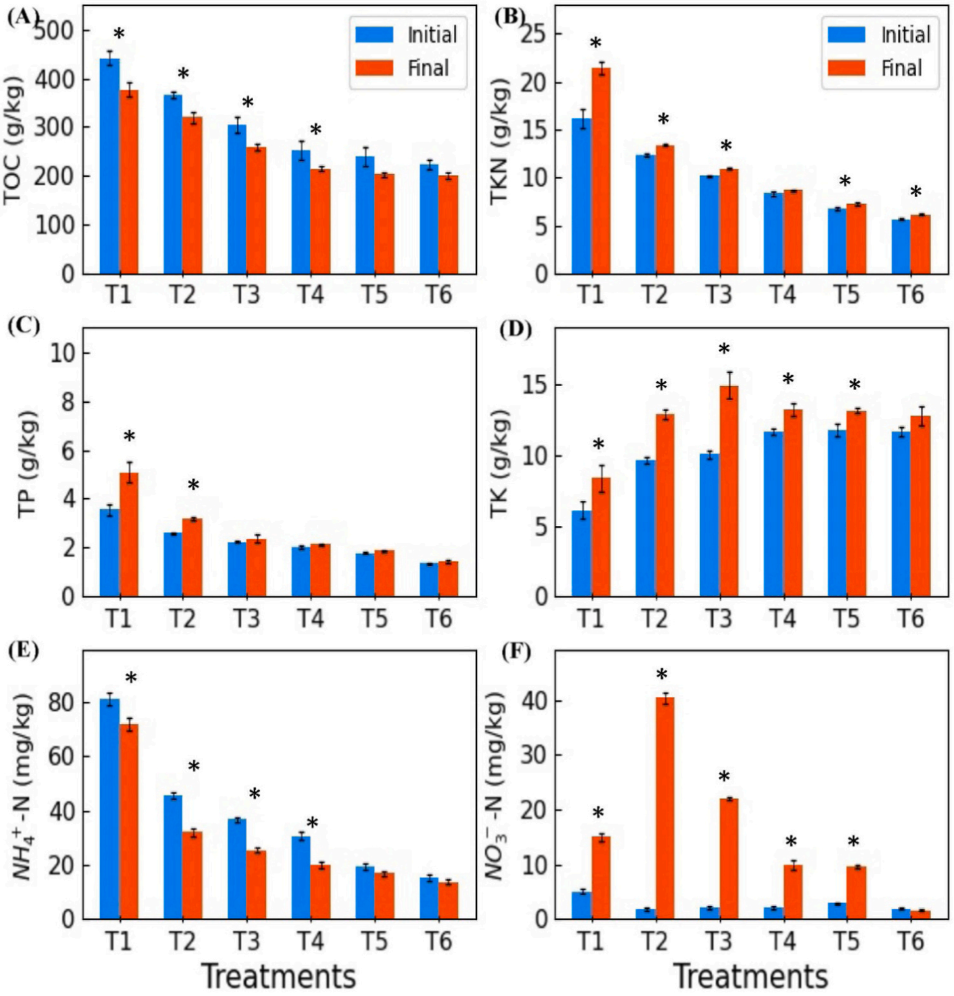

Fig. 2. Changes of physicochemical characteristics in different feed mixtures after 6 weeks of vermicompost (Mean ± S.D., n = 3). The asterisk (*) indicates a significant difference between the initial feed mixtures and final vermicompost from each treatment by Tukey's *t*-test at $P < 0.05$.

reduction of readily biodegradable organic nitrogenous compounds (Hanc and Chadimova, 2014).

EC is the measurement of total soluble salts produced during the vermicomposting process and used as an index of fertilizer quality for plant production (Awasthi et al., 2014). The initial EC values of feed mixtures were ranged from 2.82 to 4.79 ds m^{-1}. The EC values of the final vermicompost were ranged from 3.65 to 5.19 ds m^{-1} and significantly higher than their initial values (Tukey's *t*-test, $P = 0.001$; Table 4). The percentage increase of EC values in the vermicompost ranged from 7.29% (T5) to 29.25% (T1) compared to their initial values, and the variation was significant among different vermireactors (ANOVA, F = 54.941, $P < 0.05$; Table 4). The increase in EC could be attributed to the degradation of organic matter and the release of different mineral salts in available forms, such as phosphate, ammonium, and potassium, etc., during the vermicomposting process (Kaviraj and Sharma, 2003). EC in the vermireactors T1 and T2 was within the non-toxic range to an agronomic application (> 4 ds m^{-1}) as suggested by Wong et al. (2001).

3.2.2. Change in total organic carbon

The total organic carbon (TOC) in all vermireactors was reduced after 6 weeks vermicomposting (Fig. 2A). The initial TOC content of the feed mixtures was in the range of 226.50–441.97 g kg^{-1} and in the final vermicompost, it reduced to 172.42–376.22 g kg^{-1} in different vermireactors. The results showed that a maximum reduction of TOC was observed in T3 (15.12%), followed by T1 (14.88%), T2 (13.36%), T4 (12.35%), T5 (12.60%), and T6 (10.47%). The reduction of TOC during vermicomposting is an important event and takes place through shared action of earthworms and microorganisms in the feed mixtures. Earthworms break down and homogenize organic waste through the muscular action of the foregut, which increases microbiological activity and dramatically alters the biological activity of the waste (Domínguez, 2004; Venkatesh, 2007). Microorganisms provide extracellular enzymes required for organic waste decomposition (Suthar, 2009b). Moreover, the utilization of carbon as an energy source for microorganisms or earthworms can cause TOC reduction from the feed mixtures. For example, lignocellulose (cow dung) produces some liable forms of hydro-soluble sugars through microbial enzymatic actions (e.g., cellulose, β-glucosidase) that can be further utilized by growing microbial communities (Rai and Suthar, 2020; Yu et al., 2020). The conversion of some part of the organic fraction of waste into worm biomass also can lead to the C loss from the feed mixtures. In addition, respiration of microorganisms and earthworms during the degradation and conversion of initial feed mixtures to stabilized end products appeared to the major mechanism for the C loss from the substrates (Gusain and Suthar, 2020; Karmegam et al., 2021). The results showed that a significant C loss was noticed in all vermireactors except T5 and T6 (Tukey's *t*-test, $P = 0.051$ and 0.152, respectively) compared with their initial contents. The higher C loss in the vermireactors with less SDF ($\leq$ 40%) due to the respiratory and assimilatory activity of earthworms and microorganisms resulting in an ideal vermicomposting environment.

3.2.3. Nitrogen, phosphate, and potassium variations

The TKN content in all vermireactors was significantly enhanced at the end vermicomposting (Fig. 2B). The initial TKN content of the feed mixtures was in the range of 5.69–16.24 g kg^{-1} and the final vermicompost, increased to 6.17–21.41 g kg^{-1} in all vermireactors. A maximum increase in TKN was noticed in vermireactors T1 (32.05%) followed by T6 (8.44%), T2 (8.18%), T3 (8.11%), T5 (6.93%), T4 (4.32%), and the variation was significant among the different vermireactors (ANOVA, $F = 16.032$, $P < 0.05$). The increase of TKN could be attributed to the mineralization of organic materials by the action of earthworms and microorganisms. Earthworms enhance the N profiles in the form of mucus, nitrogenous excretory substances, body fluid, decomposed tissue, and growth-stimulating hormones produced during the fragmentation and digestion of organic waste in the vermicomposting process (Bhat et al., 2015). Microbes together with earthworms improve the generation of important enzymes involved in the N-mineralization process (e.g., invertase, protease, amylase, urease, and cellulose) and increase the N-fixing bacterial populations in vermicomposting systems, resulting in a further increase in N levels (Suthar, 2007; Fu et al., 2015a). As compared with their initial content, a significant increase in TKN content was found in all vermireactors except T4 (Tukey's *t*-test, $P = 0.064$). The rise of N levels in the feed mixtures T2 and T3 (containing less SDF) might be reckoned on a higher N-mineralization rate mediated by earthworms and microorganisms. However, the significant increase in TKN contents in vermireactors T5 and T6 (containing more SDF) may due to higher earthworm mortality (T5 = 26.67% and T6 = 47.78%; Fig. 2B), the decomposing tissue of lifeless earthworms enriched the N profile in vermicompost products. The results showed that the increase of TKN in vermireactor T1 (32.05%) significantly higher than that in the other vermireactors (T2-T6), which may be attributed to the primary N levels of the waste in the initial feed mixtures and the extent of waste degradation in the system (Suthar, 2009a).

The vermicomposting process demonstrated the positive role of earthworms in enhancing the N mineralization of organic waste, whereby ammonium is maintained in the nitrate form. Nitrate nitrogen (N-NO$_3^-$) content in the final vermicompost experienced an upturn trend. The initial N-NO$_3^-$ the content of the feed mixtures was in the range of 1.76–5.12 mg kg^{-1} and in the final vermicompost, it decreased to 1.60–40.37 mg kg^{-1}. The maximum increase in N-NO$_3^-$ content was observed in vermireactor T2 (22.85-fold) followed by T3 (10.08-fold), T4 (4.55-fold), T5 (3.25-fold), T1 (2.91-fold), and T6 (0.84-fold), and the variations were significantly different among all vermireactors (ANOVA, $F = 65.675$, $P < 0.05$, Fig. 2F). The reason may be due to the nitrification process promoted by earthworms and microorganisms during the vermicomposting (Bernal et al., 2009). Conversely, ammonium nitrogen (N-NH$_4^+$) contents in tall vermicompost showed an absolute downturn trend (Fig. 2E). The initial N-NH$_4^+$content was in the range of 15.41–81.11 mg kg^{-1} and in the final vermicompost, it decreased to 13.90–72.04 mg kg^{-1}. A profound decrease in N-NH$_4^+$ content was observed in vermireactors T4 (30.76%) followed by T3 (30.76%), T2 (29.52%), T1 (12.53%), T5 (11.18%), and T6 (9.07%). The significant difference in N-NH$_4^+$ level was observed in all vermireactors except T5 and T6 (Tukey's *t*-test, $P = 0.164$ and 0.259; respectively) when compared with their initial value. Most likely, the drastic drop in N-NH$_4^+$ can be attributed to the occurrence of aerobic ammonium oxidation altered by earthworms, which promoted microbial populations (e.g., nitrifying-bacteria) responsible for the accumulation of N-NO$_3^-$ (Fu et al., 2015b). Deamination of amino acids oxidative of proteins has been reported as another reason for ammonium decline (Moriskai et al., 1989). In addition, NH$_3$-volatilization also plays a certain role in the ammonium decline during vermicomposting.

The worm-worked feed mixtures after 6 weeks exhibited an increase in total phosphorous (TP) compared with their initial contents (Fig. 2C). The initial TP contents of the feed mixtures were in the range of 1.34–3.92 g kg^{-1} and in final vermicompost, it increased to 1.43–5.09 g kg^{-1}. There was about 1.18% (T4) to 29.59% (T1) increase in TP contents compared with their initial contents, and the variations were significant among all vermireactors (ANOVA, $F = 31.693$, $P < 0.05$). The increment in TP contents during the vermicomposting may be attributed to the P-soluble microorganisms in the gut of earthworm, the involvement of phosphate and phytase enzymes, and mineralization of the waste through the combined activity of earthworms and microorganisms (Deepthi et al., 2021). In addition, the initial feed mixture content also plays a major role in the final TP contents. The results showed that a significant increment of TP contents in T2 (30.18%) and T1 (29.59%), which significantly higher than other vermireactors, could be attributed to the higher TP in their initial substrates and the combined activity of earthworms and microorganisms.

The TK content is one of the important factors which determined the fertilizing property of vermicompost and it is evident that earthworms increase the nutrients by promoting the conversion of potassium to plant-available forms (Karmegam et al., 2019). In the current study, TK contents showed an upward trend in all vermireactors after 6 weeks vermicomposting (Fig. 2D). The TK content in initial feed mixtures was in the range of 6.09–11.74 g kg^{-1} and in the final vermicompost, it increased to 8.38–14.93 g kg^{-1}. A profound increase in TK contents was noticed in vermireactor T3 (48.72%) followed by T2 (37.61%), T1 (37.41%), T4 (13.47%), T5 (12.01%), and T6 (9.93%), and the variations were significant after 6 weeks vermicomposting (ANONA, $F = 29.011$, $P < 0.05$, Fig. 2D). The more increase in the vermireactors with less SDF (T1, T2, and T3) may be due to the activity of earthworm, which modifies the physical and chemical structure of the waste through feeding and enzymatic actions of glut-flora thereby enhance the conversion of available forms of potassium. Furthermore, the increases in the population of microorganisms caused by earthworm activity such as K-solubilizing microbial communities also can accelerate the conversion of available forms of potassium (Paul et al., 2018; Karmegam et al., 2019).

The relative efficiency of nutrient recovery in vermicompost calculated by different treatments is a key indicator for nutrient recovery in unit time in terms of N, P, and K as compared to the control (T1). The vermicompost obtained from most of the treatments showed TN, TP, and TK contents above the minimum levels of 1.0, 0.8, and 0.8 respectively for vermicompost following the Fertilizer Control Order 2009 (FCO, 2009). In the present study, spent drilling fluid significant decrease ($P < 0.05$) the nutrient recovery rate of TP ($F = 7.844$, Fig. 3B) and TK ($F = 20.069$, Fig. 3C) in final vermicompost in a concentration-depend manner, but not in the TKN ($P > 0.05$, Fig. 3A). Among the macronutrients analyzed, the higher relative efficiency of recovery rate for TP in final vermicompost was observed in T2 (1.01), followed by T3 (0.84), T4 (0.83), T6 (0.82), and T5 (0.80) (Fig. 3B). The higher relative efficiency of recovery rate for TK was observed in T3 (1.08) and T2 (1.00), followed by T4 (0.83), T5 (0.81), and T6 (0.80) (Fig. 3C). The higher relative efficiency of recovery rate for TP and TK in the treatment T2 and T3 indicates the effective vermiconversion of organic wastes materials and is mainly due to the organic material decomposition occurred through combined activity of earthworm and microflora (Karmegam et al., 2021). Besides, high TK content in SDF also contributed to the higher K recovery. Conversely, the relative efficiency of recovery rate of TKN in the final vermicompost was ranged from 0.79 (T4) to 0.82 (T6), and the values were lower than the minimum levels of 1. The low recovery rate of TKN may be due to the lower TKN content in the SDF, which significantly lower than that in cow dung (Table 1).

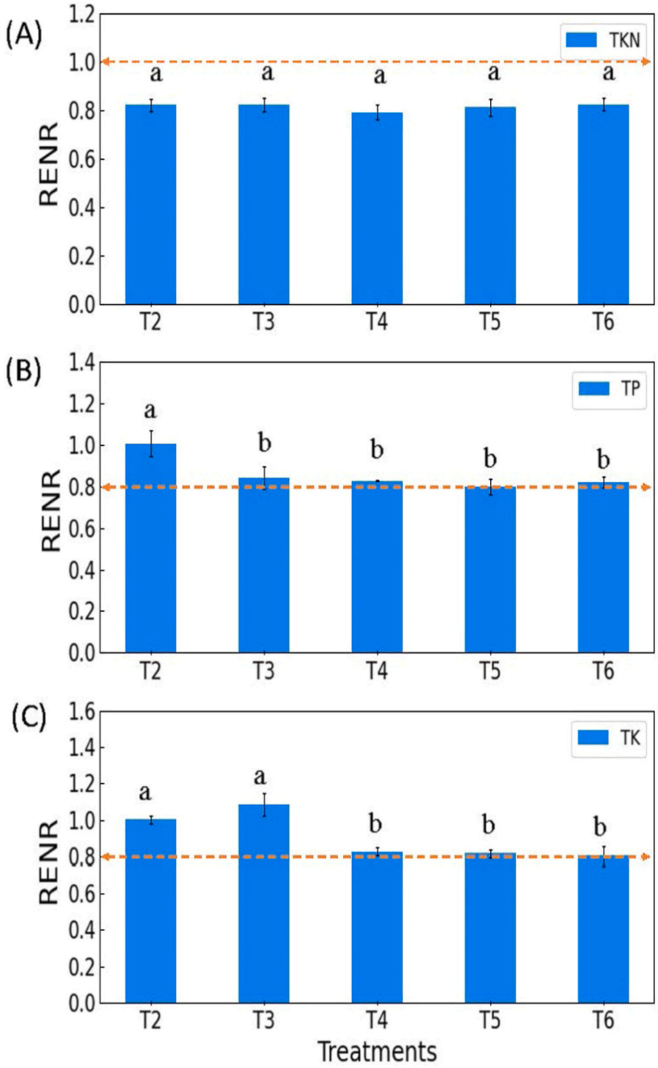

Fig. 3. The RENR (relative efficiency of nutrient recovery) of the final vermicompost for TKN (A), TP (B), and TK (C). The horizontal line indicates the minimum levels (1.0, 0.8, 0.8 for TKN, TP, TK, respectively) for most vermicompost in accordance with the Fertilizer Control Order 2009. Different lowercase letters in each column represent significant differences among all treatments, at the $P < 0.05$ level, based on the Tukey-HSD.

3.2.4. Maturity of vermicompost

The values of C/N ratios in all vermireactors showed a remarkable reduction after 6 weeks vermicomposting (ANOVA, $F = 19.778$, $P < 0.05$; Table 4). The initial C/N ratio was ranged from 27.24 to 38.50 among different vermireactors. As evident from Table 4, the final C/N ratios were ranged from 17.56 (T1) to 32.49 (T6), corresponding to an overall decrease of 15.24% (T6) to 35.48% (T1) from their initial levels after 6 weeks of vermicomposting. Other authors have observed similar trends during organic amendments with vermicomposting (Alidadi et al., 2016). The C/N ratios decline was expected due to the organic C loss as CO_2 through microbial respiration during the vermicomposting process and also production and disposal of nitrogenous substances such as a higher proportion of TKN in the final vermicompost added by the combined action of worms and microflora. The drift in C/N ratios is the criterion traditionally used for the assessment of the efficiency of determining the vermicomposting process and vermicompost maturity. A decline in C/N ratios below 20 reflects a satisfactory degree of maturity of organic wastes. Thus after 6 weeks, the vermicompost of T1 is considered as mature vermicompost with the proportions of 17.56. However, the C/N ratio in the vermireactors with SDF were slightly higher than 20 at the end of the vermicompost. This suggested that the bioconversion efficiency is largely associated with the initial carbon and nitrogen in the feed mixtures used for vermicomposting.

The N-NH$_4^+$/ N-NO$_3^-$ ratio has been established as another maturity index for composts of various organic waste, such as poultry manure, and sewage sludge (Bernal et al., 1998). Organic waste is considered ready to be used as compost when the N-NH$_4^+$ concentration decreases and N-NO$_3^-$ appears in the composting product. In the current study, the vermicomposting process decreased the N-NH$_4^+$ contents and increased the N-NO$_3^-$ contents in the final vermicompost compared to their initial values. The reduction in N-NH$_4^+$/ N-NO$_3^-$ ratio ranged from 12.65% (T6) to 97.05% (T2), and the variation was significant among different vermireactors (ANOVA, $F = 16.032$, $P < 0.05$; Table 4). When compared to the initial values, a significant decrease in the N-NH$_4^+$/ N-NO$_3^-$ ratio was observed at all vermireactors except T6 (Tukey's *t*-test, $P = 0.161$). According to California Compost Quality Council (2001), if the amount of this ratio is in the range of (0.5–3) or > 3, it will be mature or immature, correspondingly (Alidadi et al., 2016). The final N-NH$_4^+$/ N-NO$_3^-$ ratio in the vermireactors T2, T3, T4, and T5 could be considered as mature vermicompost with the amount of 0.79, 1.15, 1.92, and 1.98, correspondingly. However, the vermireactor T1 was immature vermicompost with 4.79, which may be attributed to the lower nitrification and aerobic ammonium oxidation mediated by earthworms and microorganisms during the vermicomposting.

The humification index (HI) is also an important indicator for evaluating the maturity of final vermicompost products. HI can describe the changes in the composition and properties of humus during the vermicomposting process and reflects the quality of the compost products. A higher level of organic material humification indicated by a smaller value of A_{472}/A_{664} in vermicompost may suggest the suitability of vermicompost product over the initial feed mixture (Zbytniewski and Buszewski, 2005). The worm-worked vermireactors after 6 weeks exhibited about 4.41% (T4) to 13.83% (T1) decrease in HI in the final

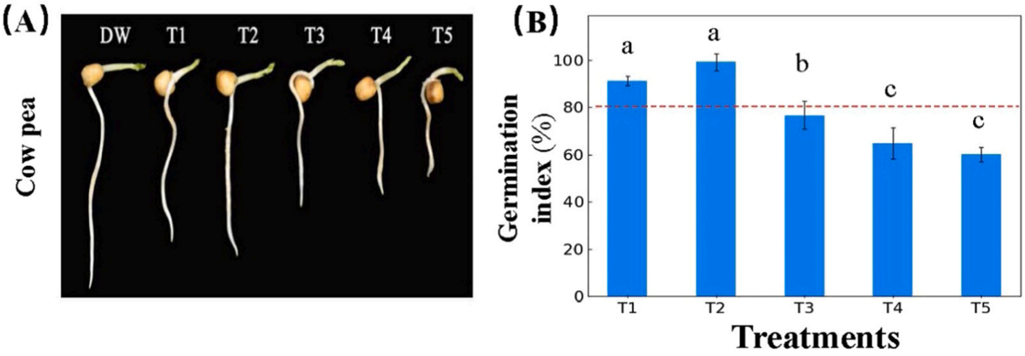

Fig. 4. Vermicompost extracts treated cowpea shows hypersensitive growth response. Growth response (A) and germination index (B) of cowpea seeds treated with DW and different extracts. Different lowercase letters in each column represent significant differences among all treatments, at the $P < 0.05$ level, based on the Tukey-HSD. The vertical line (-) represents the phytotoxicity free-range (80%).

vermicompost compared with HI in the initial feed mixtures and the variation was significant among all vermireactors (ANOVA, $F = 15.243$, $P < 0.05$; Fig. S1). A significant difference was observed in the vermireactors that contained less SDF ($\leq$ 40%) in the feed mixtures (Tukey's *t*-test, $P < 0.05$). Similar observations have been reported for the vermicomposting of paper mill sludge with *Eisenia fetida,* which decreased the HI of the initial feed mixture (Boruah et al., 2019). The lower HI in vermicompost could be attributed to the decomposition and stabilization of organic waste accelerated by the combined association of earthworms and microorganisms.

3.2.5. *RadViz visualization and VizRank*

RadViz is a nonlinear multidimensional visualization technique that can display data on three or more attributes in a 2-dimensional projection. VizRank can automatically rank visual projections of class-labeled data by their potential interestingness by showing well-separated different class values (Leban et al., 2005). In this article, VizRank was used to evaluate 4344 projections that included exactly five attributes from all attributes, and the first-ranked RadViz visualization employing five attributes (TOC, EC, TKN, C/N ratio, and HI) extracted from the attribute set is shown in Fig. S2A. The order of these attributes creates a visualization that shows two perfectly separated classes (the initial feed mixture and the final vermicompost product) (score = 99.47%). From the positions of the points in this figure, we can observe that the examples of the final vermicompost are close to the position of the attribute EC and away from the attribute C/N ratio. The attributes that appear in the top-ranked projections are expected to be those that hold the most information concerning class separation (Mramor et al., 2007). We implemented a so-called attribute ranking which is the number of appearances of the attributes in the 100 top-ranked projections, to assess the overall utility of attributes. A histogram including the scores of all attributes is shown in Fig. S2B and colored according to the different example classes with higher values for those attributes. As depicted in this graph, the C/N ratio is the most important attribute, present in almost half of the top-ranked projections, in terms of separating the initial feed mixtures. The next highest-ranked attribute is EC for the post-vermicompost products, which means that the vermicomposting process may primarily affect the EC and C/N of the initial feed mixtures.

3.3. *Enzyme activities*

The monitoring of enzyme activities during the composting process provides useful information about the decomposition of organic matter and nutrient transformation and may provide information about the maturity of compost products. The changes in hydrolytic enzyme activities throughout vermicomposting are reported in Fig. S3. In the current study, the activity of the enzyme UA and ALP showed a progressive increase during the first 2 weeks with variations among different vermireactors and then started declining by the end of vermicomposting (6th week). The UA catalyzes the hydrolysis of urea to CO_2 and NH_4^+, which is closely linked to the decomposition of organic waste (Vargasgarcia et al., 2010). The UA activity in all vermireactors was ranged from 20.93 (T6) to 41.80 (T2) mg NH_4^+ g^{-1} dw h^{-1} on the 14th day of the experiment. The maximum increase in UA activity was observed in vermireactor T3 (106.74%), following by T2 (82.18%) , T1 (63.37%) , T5 (59.98%), T5 (35.00%), and T6 (11.29%), when compared with their initial values. The increase in UA activity during the first 2 weeks was probably a consequence of the decrease in the initial content of NH_4^+ in the feed mixtures and enhanced decomposition and mineralization promoted by earthworm and microflora activities. The UA activity was ranged from 6.59 to 17.43 mg NH_4^+ g^{-1} dw h^{-1} in the final vermicompost, and the values were significantly lower than the initial levels ($P < 0.05$, Fig. S3A). The decline in UA activity in all vermireactors may be attributed to the depletion of readily biodegradable compounds at the end of vermicomposting (6th week).

Phosphatases are responsible for hydrolyzing organic P compounds into inorganic phosphate forms, and their activity depends on the nature of the initial substrate and on the rate of vermicomposting (Busato et al., 2012). In the current study, the peaked ALP activity was noted on the 14th day after vermicomposting. The ALP activity in the vermicompost ranged from 11.11 to 32.09 mg PNP g^{-1} dw h^{-1}, and changed to 37.15–48.74 mg PNP g^{-1} dw h^{-1} on the 14th day. In this period, a profound increase in ALP activity was noted in vermireactors T2 (23.18%) followed by T3 (17.51%), T1 (11.59%), T4 (7.11%), T5 (3.06%), and T6 (1.03%) when compared with their initial activities. The ALP values showed statistically significant differences among the different vermireactors ($F = 3.495$, $P < 0.05$; Fig. S3B). The increase in ALP activity during the first 2 weeks could be attributed to the initial P load (organically-bounded), earthworm activities, gut-cellular enzymes secreted by earthworm (Gusain and Suthar, 2020).

3.4. *Seed germination and early growth of cowpea*

Vermicompost quality, such as stability and maturity, should be checked before the application of post-compost product on land. Seed germination is generally used to evaluate the degree of maturity and phytotoxicity of vermicompost products for agricultural use. In the current study, the vermicompost extracts inhibited the seed germination of cowpea (Table 5). The maximum germination percentage (GP) at 4-days of cowpea was found in vermicompost extracts T2 (90.00%) followed by, T1 (86.00%), T3 (79.00%), T4 (72.00%), and T5 (72.00%). The significant inhibition in GP was observed in all vermicomposts except T1 and T2 (Tukey's *t*-test, $P = 0.060$ and 0.455, respectively) when compared with DW (92.00%). The extract-treated seedlings exhibited a hypersensitive inhibition in the primary root length and elongation of hypocotyl length. The maximum primary root length was

Table 5
Vermicompost extracts on the germination, primary root length, and hypocotyl length in 4-days-old cowpea seedlings grown under DW and the vermicompost extracts. Different lowercase letters represent significant differences among all treatments, at the $P < 0.05$ level, based on the Tukey-HSD.

Treatments	Germination percentage (%)	Primary root length (cm)	Hypocotyl length (cm)
T1	92.00 ± 2.45a	6.02 ± 0.72a	2.58 ± 0.23a
T2	86.00 ± 4.89ab	5.91 ± 0.58a	1.98 ± 0.37b
T3	90.00 ± 4.47a	6.10 ± 0.55a	2.37 ± 0.15ab
T4	79.00 ± 4.89bce	5.24 ± 0.47b	1.92 ± 0.35bce
T5	73.00 ± 4.05c	4.92 ± 0.57bce	1.63 ± 0.23c
T6	72.00 ± 4.10c	4.62 ± 0.63c	1.58 ± 0.30c

noticed in the extracts T2 (6.10 cm), followed by T1 (5.91 cm), T3 (5.24 cm), T4 (4.92 cm), and T5 (4.62 cm). The significant inhibition ($F = 105.434$, $P < 0.05$) in primary root length was observed in all extract-treated seedlings except T1 and T2 (Tukey's *t*-test, $P = 0.726$ and 0.794, respectively) when compared with that in DW (6.02 cm). The maximum hypocotyl length s was detected in extracts T2 (2.37 cm) followed by T1 (1.98 cm), T3 (1.92 cm), T5 (1.53 cm). A significant inhibition in hypocotyl length was observed in all vermicompost extracts when compared with DW (2.58 cm) ($F = 18.854$, $P < 0.05$). Higher inhibition on plant growth can be attributed to ion toxicity such as high levels of Na^+, Cl^-, SO_4^{2-} and other soluble salts in the SDF, which leads to osmotic and oxidative effects and further causes cell death.

Germination index (GI) which combines the measure of seed germination and root growth of seeds is one of the most sensitive biological parameters to assessed the phytotoxicity of vermicompost products and the GI values exceeding 80% indicates a better vermitransformation. In the current study, a maximum GI was observed in treatment T2 (98.74%) followed by T1 (91.43%), T3 (76.67%), T4 (64.09%) and T5 (60.29%), and the showed significant differences among different treatments ($F = 71.240$, $P < 0.05$; Fig. 5B). The higher GI in treatment T2 could be attributed to the phytotoxicity-free nature of vermicompost as well as the presence of high levels of plant nutrients such as TKN, TP, and TK. In contrast, the GI in treatments T3, T4, and T5 showed lesser than 80% in the final vermicomposting, which indicates the higher phytotoxicity may be attributed to the lower activity of earthworm by the adverse environmental conditions such as high pH and EC, high salty, and poor nutrients in the final vermicompost.

3.5. Genotoxicity assay

Although vermicomposting is beneficial for industrial sludge because it reduces their toxicity, genotoxicity assessment of vermicompost products is also necessary to ensure the safety of post-vermicompost products before use in agriculture. The use of various plant bioassays to monitor the toxicity of industrial sludge is well known in the literature. In the current study, genotoxic effects of the vermicompost extracts were assessed in cowpea by the mitotic index (MI) and chromosomal abnormality (CA), reliable parameters for identifying genotoxicity, and the toxicity levels were assessed by the increase or decrease in MI and the types of CA (Leme and Marinmorals, 2008). Fig. 4 summarizes the effects of different extracts on MI and CA in the root meristematic cells of cowpea. The MI value was calculated from the total number of dividing cells in the cell cycle. The MI decreased significantly with increasing concentrations of SDF in the vermicompost ($F = 25.396$, $P < 0.05$, Fig. 5I); The MI of the control (DW) was 13.52% and the MI of the vermicompost extracts was in the order: T1 (11.38%) > T2 (11.25%) > T3 (9.13%) > T4 (8.13%) > T5 (7.38%). There is no significant difference was observed in T1 and T2 (Tukey's *t*-test, $P = 0.063$ and 0.053) when compared with the control (DW). Various forms of CA covering most of the mitotic stages were observed in presence of a higher concentration of SDF in vermicompost (T4 and T5). The CA significantly increased with increasing concentrations of SDF in vermicompost ($F = 86.052$, $P < 0.05$, Fig. 5J). The abnormality index of the control (DW) was 5.46%, and the value of vermicompost extracts in the order: T1 (8.56%) < T2 (10.34%) < T3 (17.63%) < T4 (26.00%) < T5 (27.75%). Micronucleus (MN) analysis, along with CA analysis, is sensitive in the detection of environmental mutagens (Leme and Marinmorales, 2008). In addition to evaluating mutagenic effects, MN analysis also enables an investigation of action mechanisms of chemical agents. In the current study, MNs and MB were found in cowpea meristematic cells when exposed to vermicompost extracts with higher SDF in the initial feed mixture (T4 and T5). Taken together, these results indicate that vermicompost extracts induce root retardation in cowpea seedlings, which might occur due to inhibition of cell division in the actively dividing root apical meristematic region. And the MN detection revealed no potential mutagenic effects of post-vermicompost product at treatment with lower SDF.

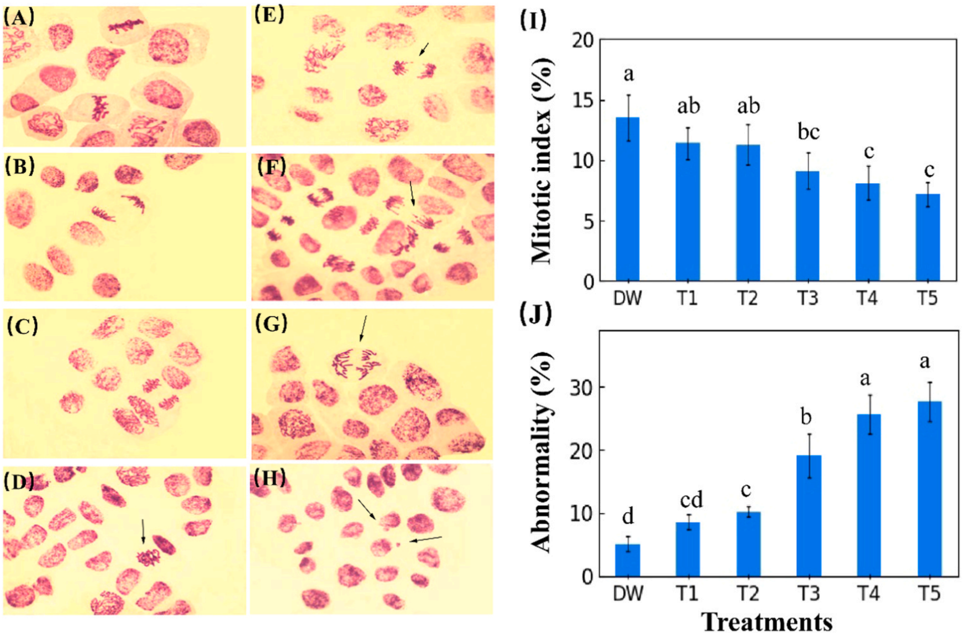

Fig. 5. Assessment of mitotic index (MI) and chromosomal abnormality (CA). Images of stages of mitosis from DW (A–C) and vermicompost extracts treated (D–H) root tip samples. Normal metaphase, anaphase, and telophase (A–C); Metaphase with chromosome stickiness (D); Metaphase with Chromosome break and bridge (E and F); Multipolar metaphase (G); Micronucleus and nuclear bud (H); Determination of mitotic and abnormality indices (%) (I and J) obtained from control and vermicompost extracts treated root tip samples. The micrographs (A–H) using the optical microscope at a total magnification of 40 × 10. Different lowercase letters in each column represent significant differences among all treatments, at the $P < 0.05$ level, based on the Tukey-HSD.

4. Conclusions

Vermitechnology can be used as a potential tool to convert spent drilling fluid into nutrient-rich vermicompost. Vermicomposted materials showed an increase in EC, total nitrogen, N-NO$_3^-$, total phosphorous, total potassium, whereas a decrease in total organic carbon, C/N ratio, N-NH$_4^+$. The final vermicompost with less spent drilling fluid ($\leq$ 30%) had a relatively lower C/N and N-NH$_4^+$ /N-NO$_3^-$ ratio indicate its maturity and agronomic potentials. Results suggest that spent drilling fluid mixed with cow dung in an appropriate ratio not only could enhance the nutrient profile of vermicompost products, at the same time supports the growth and reproduction of earthworm in vermireactors. Also, seed germination, mitotic index, and chromosomal abnormality assay using cowpea highlight that the final vermicompost T2 is lower phytotoxicity and genotoxicity for agricultural use. This study provides a sound basis for that vermicomposting can be as a potential technology to convert the drilling waste into value-added material for low-input basis sustainable environmental management.

CRediT authorship contribution statement

ZW, ZC, YN, and MH planned and designed the research and experiments. ZC analyzed and interpreted the data regarding difference in the physiological parameters. ZW performed the experiment of cytology, and was a major contributor in writing the manuscript. PR provide the drilling waste and the information about the production of the drilling waste. MH acquired the fund for the study and helped to review the manuscript. All authors discussed the results, read and approved the final manuscript.

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.

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Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.ecoenv.2021.111994.

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