Enlo. II/Annexure I: Final Technical Report

1.1 Introduction

Municipal solid waste (MSW) is an inescapable byproduct of human activities which is growing at an alarming rate, in many cases even faster than the growth of urbanization (Singh et al., 2011; Hoornweg and Bhada-Tata, 2012; Ali et al., 2014). Although, environmental and managerial problems with the ever-increasing trend of MSW generation have been felt throughout the world, it is the developing countries which are the most vulnerable. India once deemed to be agro-prime nation has undergone a sea change in the past two decades that is evident from the sound infrastructure development attained lately (Agrawal, 2015). Several small townships have been rapidly transformed into big cities in India that has resulted in considerable rise in problems related to solid waste generation as a whole in India. However, the characteristics of municipal solid waste (MSW) are highly heterogeneous; vary widely among cities depending on the level of industrialization and living standard of the inhabitants (Zhang et al., 2008). In most of the developing nations including India, the MSWs are generally dumped in low lying areas without pertaining to the guidelines of sanitary landfilling (Naryana, 2009; Keisham and Paul, 2015). Consequently, solid waste generation is closely linked with environmental pollution. The leachates produced land disposed solid wastes also greatly contaminate soil, surface water, and groundwater via dissolution of toxic metals and other pollutants. On the other hand, solid wastes are good source of nutrients which can be harnessed for agricultural application. Therefore, it is imperative to find out eco-friend solutions for converting solid wastes into nutrient rich products.

In this context, vermicomposting is a feasible and profitable recycling avenue for MSW, considering the dominance of biodegradable substances in the waste composition. Vermicomposting has emerged as a desirable option to convert various kinds of solid wastes into valuable organic fertilizer (Suthar et al., 2010). Generally, epigeic or surface dwelling earthworms are utilized as bio-agents for rapid and efficient decomposition of various industrial and organic wastes (Bhattacharya and Kim, 2016). Earthworms have unique survival strategies in stressful condition owing to their rapid multiplication and feedstock stabilization efficiency. The digestive tracts of earthworms harbor numerous beneficial microorganisms and their extracellular enzymes which facilitate rapid mineralization of the feed materials. Eventually, mineralized substances, microorganisms, and enzymes are readily released through worm excreta and thereby carry out the degradation of feedstocks along with the earthworms.

Interestingly, earthworms are known for their unique bioaccumulation potential for different toxic metals (Cd, Pb, Hg, and Zn) (Song et al., 2014). The bound metals are stored in insoluble

forms in the chloragogenous tissues, situated in the posterior end of the earthworm body and remain inert for long time even after the worms die (Goswami et al., 2016; Roux et al., 2016). Generally, it is explained that the entry of toxic metals into the earthworm intestines induces metallothionein (MT) expression. This cysteine rich metal chelating protein has been vastly reported in various invertebrates (Hockner et al., 2011; Hockner et al., 2015). Although majority of the literatures agree on the fact that the metal binding in earthworm intestines is mediated by MTs, differing findings indicated revealed that there are also alternative metal binders, such as phytochelatins (Liebeke et al., 2013). Moreover, induction of MT often does not correlate with the accumulated concentration of metals in the earthworm intestines (Goswami et al., 2016).

Under these perspectives, in the present project, performance of few earthworms in transforming wide ranges of solid wastes into valuable organic manure through vermicomposting has been comprehensively evaluated and large scale applicability of the vermicompost was assessed in comparison with biochar for maize cultivation. Moreover, we made an effort to understand the metal binding properties in *Eisenia fetida* using fluorescent probed metals (Cd and Zn) and searched the signature of some non-metallothionein high molecular weight metal-inducible proteins in earthworm guts. The fluorescent based technique also facilitated to estimate the apportionment of metals in earthworm body and vermicompost.

1.2 Objective of the study:

i. To navigate the in situ movement of fluorescent tagged heavy metals in earthworm and in the earthworm cast in a vermicomposting system.
ii. Exploring metal binding potential of earthworm (*Eisenia fetida*) through fluorescent tagging and identification of novel metal binding protein in earthworm intestinal cells.
iii. To evaluate the metal detoxification potential of the tested earthworm species in metal contaminated municipal solid waste (MSW) through small scale laboratory experimentation.
iv. To assess the efficiency of the tested earthworm species for large scale production of a ‘clean vermicompost’ and to evaluate the impact of the vermiremediated MSW on crop production and quality in Assam.

2. Materials and Method

2.1 Layer wise MSW characterization

A) FTIR spectroscopy and SEM imaging: The layer differentiated MSW samples (L1, L2, and L3) were mixed with KBr in 1:8 ratio (w/w) and pressed hydraulically to form pellets. The pellets were scanned in mid infrared wavelength (4000-400 cm^{-1}) in FTIR spectrophotometer

(Nicolet Impact 410, USA) as described by (Gupta and Garg, 2008). Scanning electron microscopy was utilized for the structural elucidation of the layer sourced MSW samples (Jeol, Japan). Images were obtained at low magnification range of 300-500 to explore the skeleton and particle architecture of the substrate.

B) Solubility experiment and geochemical modeling (Visual MINTEQ): The study was performed following (Bhattacharyya et al., 2011; Goswami et al., 2013). Briefly, the L1, L2, and L3 samples were suspended in deionized water in the ratio 1:10 (w/v) in conical flasks and kept for continuous shaking on a mechanical shaker at 120 rpm for one month. Periodic samples were collected at a regular interval of 10 days (0.10, 20, 30) filtered and analyzed for various physico-chemical properties. The toxic impacts were ascertained by computing relative mobility from the ratio of the concentration of bioavailable form of a metal and the total concentration of that metal in the substrate. The relative mobility of the heavy metals from each layer was measured using the equation no. 1:

$$Relative\ mobility = \frac{Concentration\ of\ the\ heavy\ metal\ in\ available\ form}{Total\ metal\ concentration} \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots (1)$$

2.2 Toxicity and epigenetic effects of MSW on earthworm: Earthworm (*Eisenia fetida*) specimens were introduced in in MSW rich feedstock under laboratory condition. The feedstocks were prepared by mixing MSW in different proportions with cow-dung. For comparison, a set of aerobic composting treatments were kept. The information on the used treatment combinations can be seen as under:

Vermicomposting	**Composting**
Only MSW+*E. fetida*	Only MSW
MSW+CD (1:1) +*E. fetida*	MSW+CD (1:1)
MSW+CD (2:1) +*E. fetida*	MSW+CD (2:1)

The incubation was carried out for 30 days under ideal conditions (Goswami et al., 2014). Samples were drawn periodically at 10 days interval and analyzed for different physico-chemical parameters (Page et al., 1982).

2.2.1. Stress Indicators: Stress enzyme assay was performed to find out the mechanism by which earthworms respond to the heavy metal stress. Earthworms subjected to different feedstocks were sacrificed for the analysis. The earthworms were collected, gut cleaned and analyzed for Catalase, Superoxide dismutase (SOD) and lipid peroxidation following suitable

protocol. Catalase assay was performed by following the method developed by Aebi (1984). SOD activity was assessed by its ability to inhibit the anti-oxidation of hematoxylin into hematin and subsequent changes in the absorbance at 556 nm (Martin et al., 1987). While, lipid peroxidation was determined according to the method described by (Dhindsa et al., 1981).

2.3. Qualitative assessment of vermicomposts using different earthworm species

Three earthen vermireactors were prepared on a parallel basis for the three different earthworm species. The vermireactors were designed according to standard sizes and dimensions [size: 3 L; dimensions: 45 cm (height) × 15 cm (base radius) × 30 cm (top radius)] with one leachate hole at the base (Hussain et al., 2018). The vermireactors were placed on a concrete-floored vermi-yard with a roof made of corrugated sheet and open sides. The collected MSW materials were thoroughly mixed with cow dung (CD) at a ratio of 3:1 after determination of their inherent physico-chemical characteristics. This substrate mixture recipe has been adopted from our previous study (Paul et al., 2018). The following feedstock combinations were used for this study:

Control – Aerobic composting with LSW+CD (3:1) feedstock (as composting control)

$VC_{Eisenia}$ – Vermicomposting of LSW+CD (3:1) with *E. fetida*

$VC_{Eudrilus}$ - Vermicomposting of LSW+CD (3:1) with *E. eugeniae*

$VC_{Perionyx}$ - Vermicomposting of LSW+CD (3:1) with *P. excavatus*

The experiment was conducted for 60 days with temperatures of 27-31 ºC and moisture contents of 40%-50%. These conditions were maintained by sprinkling water on the reactors and turning the pile twice daily at 9 am and 4 pm.

2.3.1 Chemical analysis

The temporal changes in the chemical properties of the bioprocessed SMS were analyzed based on the variations in pH, total organic carbon (TOC), total Kjeldahl nitrogen (TKN), and available P according to standard protocols (Page et al., 1982).

2.3.2 Microbial community analyses

The PLFA analysis was performed to study the microbial community structure in the selected vermicomposted samples. The selection of vermicomposted samples for PLFA assay was done on the basis of the results obtained from microbial counts, Mq, and qCO_2. The detailed procedure of PLFA assay has been described in our previous paper (Hussain et al., 2018).

2.4 Fluorescence tagging and identification and purification of metal binding protein

We selected *Eisenia fetida* as test organism because of its wide acceptability as a vermicomposting agent and consistent metal removal efficiency (Goswami et al., 2016). The taxonomic position of the worm specimens was confirmed with the help of the Zoological Survey of India. Then, the selected earthworm specimens were exposed to known concentration of previously synthesized Cd and Zn fluorescent compounds as detailed in our earlier article (Goswami et al., 2016). Our aim was to search some hitherto unknown metal binding proteins and we hypothesized that the proteins could be larger than 100 kDa based on our previous findings (Goswami et al., 2016). Initially, the protein contents in worm body were measured following Bradford (1976) and freeze-killed earthworm specimens were homogenized with 1X PBS and subsequently centrifuged for 10 minutes at 10000×g. The supernatants were collected and subjected to Amicon YM-100 filter devices (Millipore, Bedford, MA) for separating out the above 100 kDa protein fractions. Then, identification and purification of metal-induced high molecular weight proteins were conducted through gel exclusion chromatography, gel electrophoresis, immunoblotting techniques (Hussain et al., 2021).

2.5 Crop trial

We have conducted the crop trial with Maize (*zea mays*) in a typical alluvial soil of Assam in Tezpur ($26^0$41'31.8" N $92^0$50'02.4"E). The vermicompost was prepared in the vermicomposting unit of Soil and Agro Bioengineering laboratory (SABE Lab) situated in the Department of Environmental Science, Tezpur University. An established method demonstrated by Paul et al., 2018 was used for preparation of the vermicompost. The feedstock was formulated by mixing solid waste and cow dung at 1:1 ratio. The particular ratio was selected based on the results of the biocomposting experiments. The mixture was then poured in vermibed and *Eisenia fetida* was added to it at the rate of 10 worms per kg. Adequate aeration and moisture were ensured by churning and watering the mixture regularly. During the incubation period, ambient temperature ranged between 24-29^{0}C. The prepared vermicompost was collected after 60 days of incubation, sieved and kept in air tight bags for field application. The bio char used in the study was prepared with rice straw in the farmer's field by the process of updraft pyrolysis, maintaining adequate anaerobic condition and temperature (500-700^{0}C) in a customized updraft drum biochar maker.

The experiment was conducted for two years. The crop was sown in February and harvested in May. This is the recommended sowing time for North Brahmaputra bank according to the Package of practices for Kharif crops in Assam (2015). A hybrid variety of

maize namely Ganga 5 was selected for cultivation. The seeds were sown at a spacing of 25 cm (seed to seed) × 60 cm (row to row). The dose of organic fertilizers, vermicompost (VC) and bio char (BC) was applied one week before sowing the seed as recommended in the Package of practice for Kharif crops in Assam (2015). The dose of inorganic fertilizes was applied on the day of sowing seed.

The entire recommended dose of inorganic P and K (SSP and MOP respectively) and 1/3 of the recommended dose of N (urea) was applied initially and the remaining 2/3 of N was applied in two equal doses. One at knee height stage (30-35 days) after planting and other at tasseling stage (45-60 days). All the treatment combinations were applied to the plots keeping the other management practices (irrigation, application of organic and inorganic fertilizers, plant protection measures, weeding, cleaning and crop protection etc.) identical during the experimental periods. The general recommended dose of fertilizer for hybrid maize crop is 60:40:40 kg N-P_2O_5-K_2O / ha. The recommended dose of inorganic fertilizer has been modified and substituted by organic inputs VC and BC. The treatment combinations used for the study is given as below:

Detail of the treatment combinations applied during the experiment

T_1	**NPK_{50}**	50% Recommended NPK
T_2	**NPK_{50} + BC**	50% Recommended NPK + BC (3 t ha^{-1})
T_3	**NPK_{50} + VCM**	50% Recommended NPK + VCM (1.5 t ha^{-1})
T_4	**NPK_{50} + VCM + BC**	50% Recommended NPK + VCM (1.5 t ha^{-1}) + BC (3 t ha^{-1})
T_5	**NPK_{100}**	100% Recommended NPK
T_6	**NPK_{100} + BC**	100% Recommended NPK + BC (3 t ha^{-1})
T_7	**NPK_{100} + VCM**	100% Recommended NPK + VCM (1.5 t ha^{-1})
T_8	**NPK_{100} + VCM + BC**	100% Recommended NPK + VCM (1.5 t ha^{-1}) + BC (3 t ha^{-1})
T_9	**NPK_{75} +VCM + BC**	75% Recommended NPK + VCM (0.75 t ha^{-1}) + BC (1.5 t ha^{-1})

2.8 Statistical analysis

We performed one-way ANOVA followed by Least Significant Difference (LSD) tests for all lab based experiments. The temporal data on various parameters (pH, CEC, TOC, TKN, CEC, HAC, FAC, DOH, available K, P and metals) were analyzed using a two-way ANOVA with three observations per cell in order to accommodate the temporal variations. For crop trial, two-way ANOVA was performed by following standard method. Finally, for identifying optimum treatment combinations, Least Significant Difference (LSD) test have been implemented.

3. Result and Discussion

3.1 Structural and functional elucidation of the layer wise sorted M

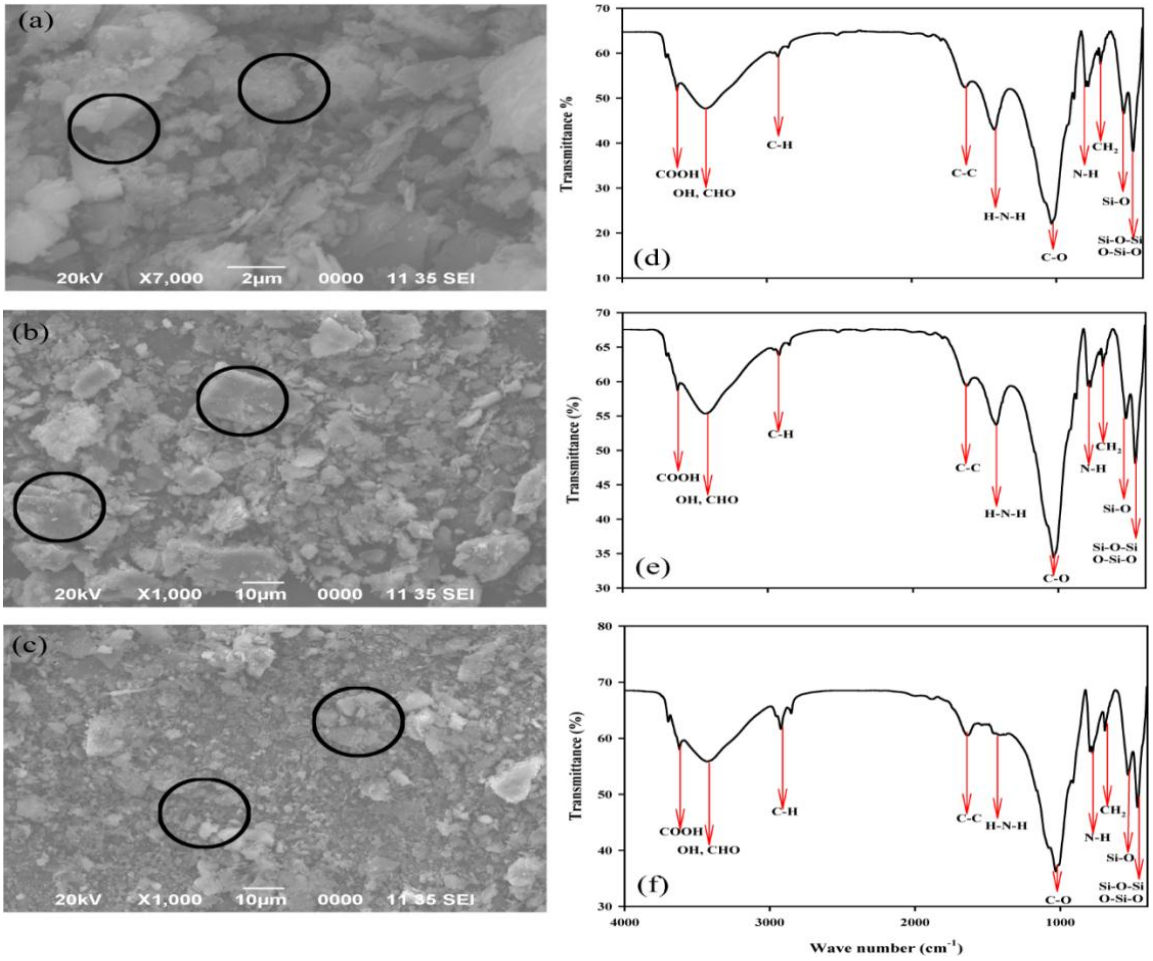


Fig. 1: (a-c) SEM images and (d-f) FTIR micrographs illustrating the structural chemical environments of the MSW samples sourced from different layers (a, d represents the top layer; b, e represents the middle layer; and c, f represents the bottom layer). The circles in the SEM images emphasize on decreasing size of the particles within the layers (L1: top layer, L2: middle layer, L3: bottom layer of the MSW heap

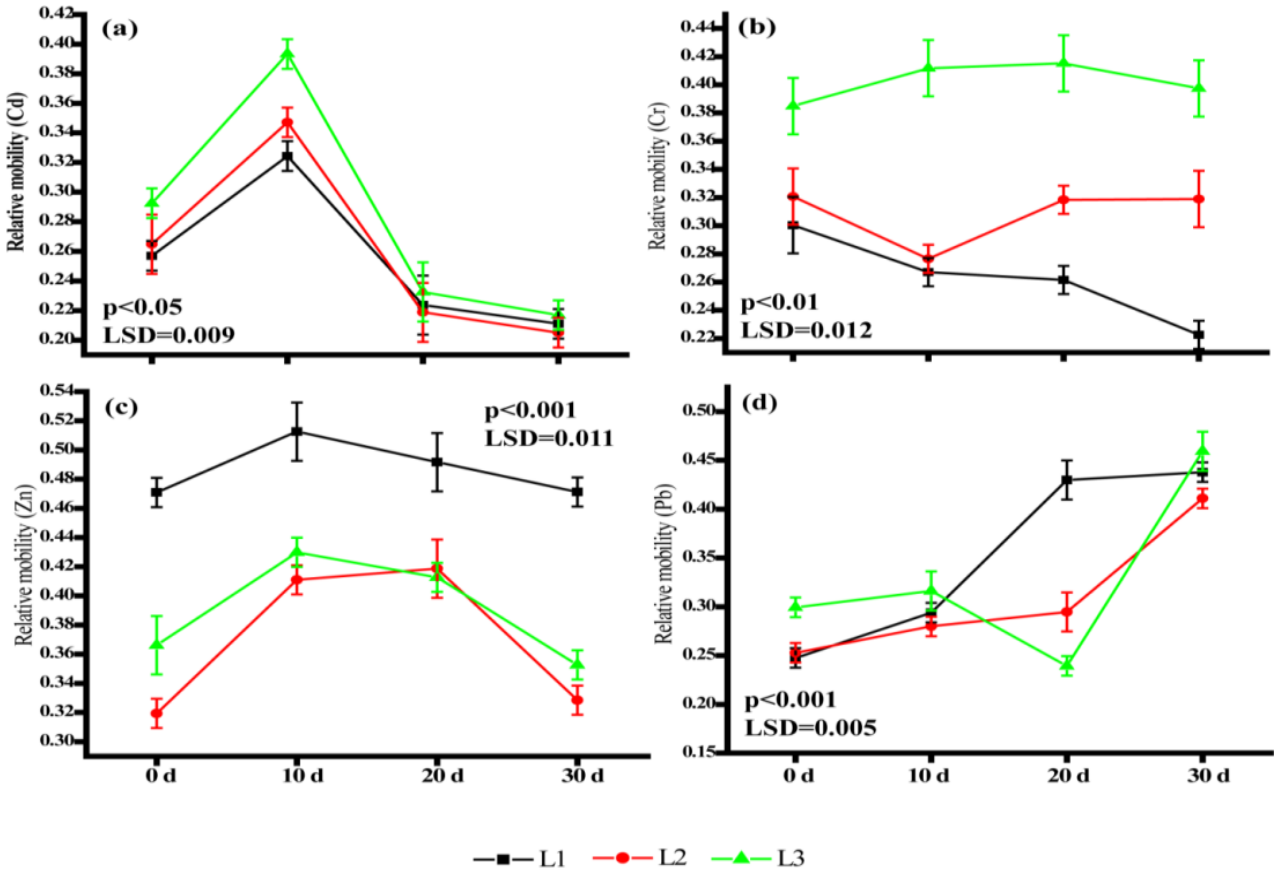
Scanning electron microscope (SEM) images exhibited some interesting results (Fig. 1a, b, c). The widely expressed variations among the MSW layers were correlated to the grain size, pore size, and the basic structure. Grain size was in the order L1>L2>L3. Both crystalline as well as amorphous structures were noted in the samples. Crystallinity was highest in L3 followed by L2 and L1 while porosity was greatest in L1 and lowest in L3 (Fig. 1a, b, c). Increase in crystallinity along the landfill depth implies that rigidity and resistibility of MSW mass are governed by the ageing factor (Kejun et al., 2011). Thus workability of the L2 and L3 fractions would materials would be difficult.

Fourier transform infrared spectroscopy (FTIR) is a useful tool for determining the chemical profile of different solid wastes (Mondal et al., 2017; Sahariah et al., 2015). A close resemblance among the spectral band of all the three layers with some variations in transmittance intensity was evidenced in this study (Fig. 1d, e, f). Transmissibility of waste materials generally varies with the decomposition state of the substrate (fresh, partially or fully decomposed) (Smidt et al., 2002). Peak in the region 3200-3700 cm^{-1} due to OH stretching was found in all samples. A band at 1637 (N-H amine) was observed in L1 whereas a band for primary amine at 3422 cm^{-1} was observed in L3. This amine is presumably an organic derivative of ammonia which are readily formed during degradation of MSW (Sahariah et al., 2015). However, the intensity of band at 700 cm^{-1} (C=C) was higher in L1 than L3. Some other bands such as C-N (Amine), a band at 1629 cm^{-1} (cyclic alkene) were evidenced in L3 but not in L1 (Fig. 1d, f).

3.2 Relative mobility of toxic heavy metals (Cd, Cr, Pb, Zn) from the MSW layers

The relative mobility [Eq. (1)] of potentially toxic metals (Cd, Cr, Zn and Pb) was significantly higher in L3 samples than L1 and L2 ($p<0.05$; Fig. 2a, b, c, d). Such mobility pattern of metals has also been reported from MSW generated in cities of developing countries like Tanzania (Kazuva et al., 2018). Greater microbial activity and enzymatic reactions possibly accelerated the decomposition process and released the toxic metals in L3 (Ayangbenro and Babalola, 2017). Although the bioavailability of Cd and Zn showed a declining trend, mobility of Pb and Cr significantly increased over time (Fig. 2a, b, c, d). When these solubility data were fed into the MINTEQ, higher saturation of metal borne minerals was predicted in L3 samples than in L1 and L2. This observation was in good agreement with results obtained for the MSW generated in Guwahati, Assam (Sahariah et al., 2015). Precipitation of Zn as $Zn_3(PO_4)_2$: $4H_2O$ was spectacular in all the samples; and its dissolution dynamics was in the order L1>L2>L3. Occurrence of Pb-rich minerals indicates higher risk of Pb mobility in the long run. The results were also in good agreement with some previous reports (Sahariah et al., 2015; Zhang et al., 2008).

Fig. 2: Relative mobility of (a) Cd, (b) Cr, (c) Zn, (d) Pb from the three MSW layers over the time scale (L1: top layer, L2: middle layer, L3: bottom layer of the MSW heap)



3.3 Role of earthworm species (Eisenia fetida, Eudrilus eugeniae, and Perionyx excavatus) on end product quality

3.3.1 Changes in pH, TOC, TKN, and P levels under different conditions

It was imperative to assess the state of organic matter decomposition with regard to certain indicative chemical parameters such as pH, TOC, TKN, and available P. The temporal changes in these attributes during vermicomposting of the SMS feedstock with the three different earthworm species are presented in Fig. 3. In general, the substrate pH decreased over time in the *Eisenia*- and *Eudrilus*-mediated vermicomposting systems ($VC_{Eisenia}$ and $VC_{Eudrilus}$) when compared with the initial values. In contrast, pH slightly increased in the Perionyx-mediated vermibeds (i.e., $VC_{Perionyx}$). Reduction in pH may be accounted for by the production of organic acid, CO_2, and nitrate during mineralization of organic matter.

The TOC of the vermibeds was reduced significantly (e.g., by 1.4- to 2.5-fold) as compared to composting systems (Fig. 3). However, the reductions in TOC were most prominent in the $VC_{Eisenia}$ system. Correspondingly, TKN levels significantly increased in the vermibeds as

compared to composting (Fig. 3). The increase in nitrogen availability in vermicomposts may be due to N-fixing microorganisms released via earthworm excreta (Hussain et al., 2016). The availability of phosphorus was significantly higher in the $VC_{Eudrilus}$ vermibeds than in the $VC_{Eisenia}$ and $VC_{Perionyx}$ ($p = 0.000$; LSD = 10.30). Recently, *E. eugeniae* was reported to have high efficiency in solubilizing large amounts of phosphorus (Paul et al., 2018).

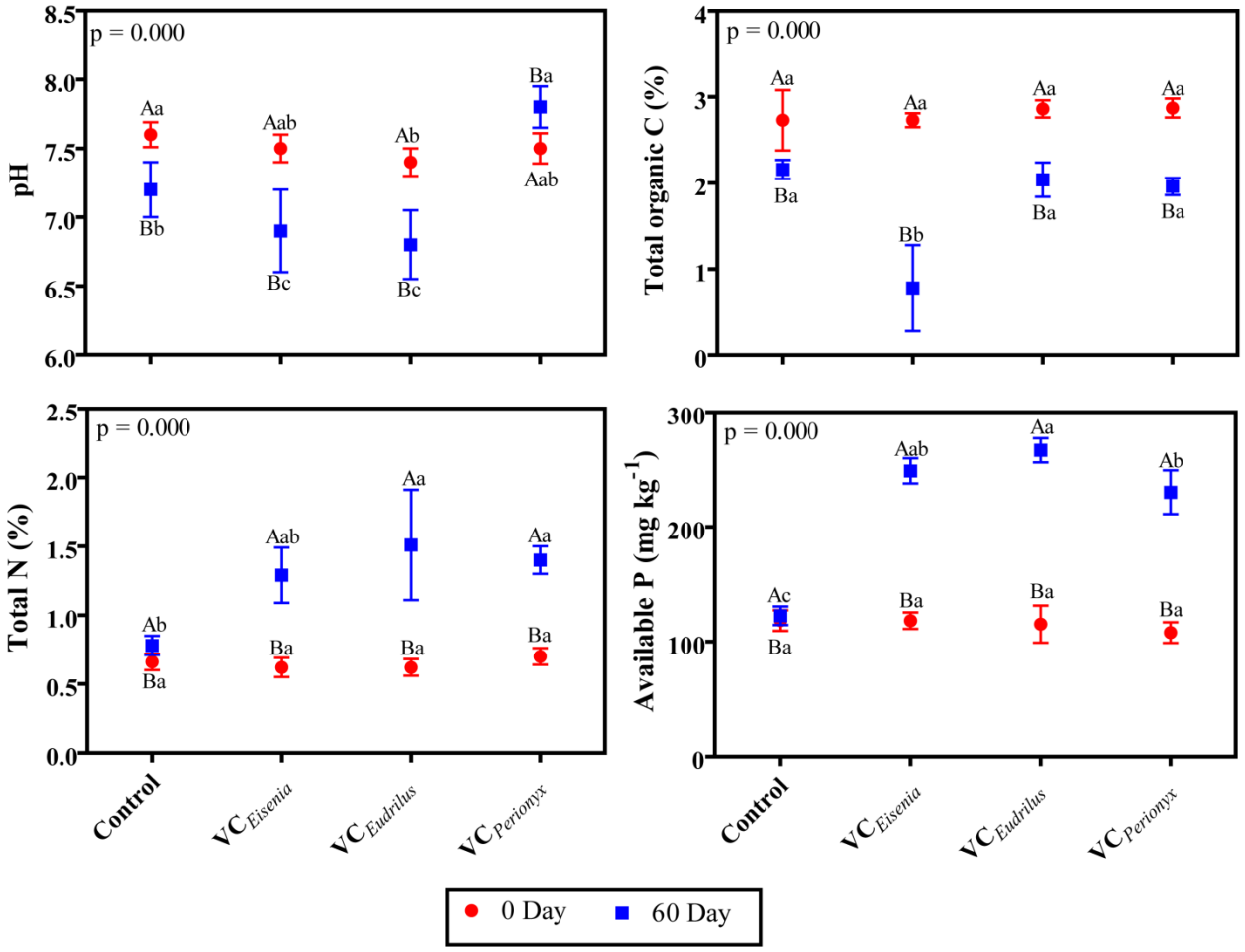


Fig. 3. Temporal variation in pH, total organic C, total Kjeldahl N, and available P of spent mushroom straw based feedstocks under the composting and vermicomposting system

3.3.2 PLFA based investigation of microbial community structure in vermibeds

The Gram-negative bacterial PLFAs were greater in $VC_{Eudrilus}$ than in the other two systems, whereas Gram-positive bacterial PLFAs were most abundant in $VC_{Perionyx}$ followed by $VC_{Eisenia}$. The occurrence of actinomycetes groups was also significantly higher in the $VC_{Perionyx}$ followed by $VC_{Eudrilus}$ and $VC_{Eisenia}$ (Fig. 4). The abundance of Gram-positive bacterial communities in the $VC_{Perionyx}$ and $VC_{Eisenia}$ signifies favorable earthworm-feedstock

compatibility in these systems. Moreover, many arbuscular mycorrhizal fungi (AMF) were observed in the $VC_{Perionyx}$ and $VC_{Eisenia}$ feedstocks. AMFs are natural biofertilizers that normally reside in the root zone soil in agricultural or forest lands (Berruti et al., 2015). Interestingly, the proportions of monounsaturated (MUFA) and polyunsaturated fatty acids (PUFA) in the total PLFA were the highest in the $VC_{Perionyx}$ and $VC_{Eisenia}$, respectively (Fig. 5). The signatures of some typical PUFA (e.g., 18:1ω9c and 18:2ω6,9c) in the PLFA profiles of the vermicomposted SMS samples revealed the presence of eukaryotes and ectomycorrhizal fungal communities in the processed materials (Quideau et al., 2016). Hence, the occurrence of AMFs, MUFA and PUFA in these SMS vermicomposts adds greater value to the sustainable recycling of agricultural waste.

Fig. 4 Phospholipid fatty acid (PLFA) identified microbial groups in the composted and vermicomposted SMS-based feedstocks at 60 days of incubation.

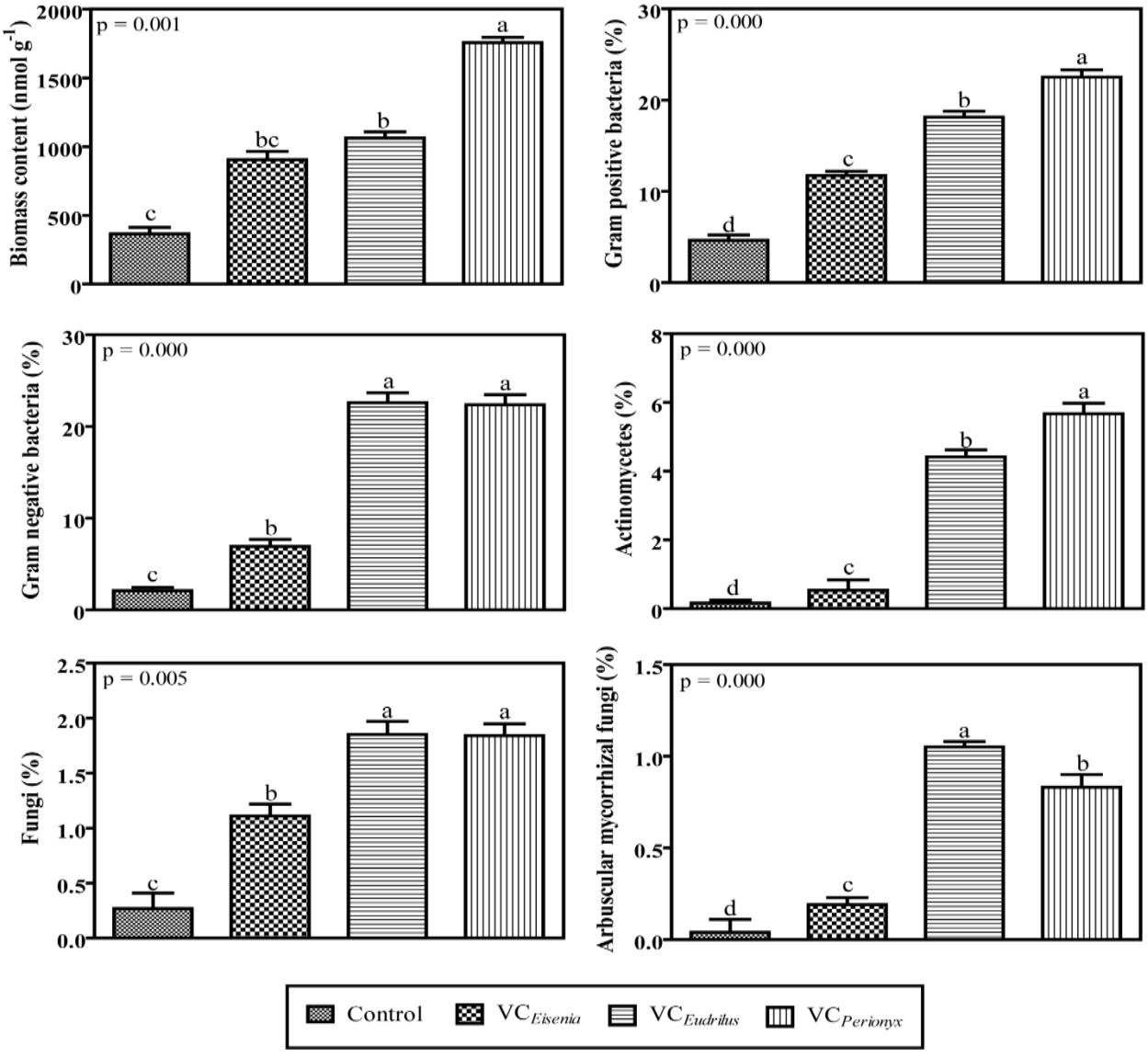
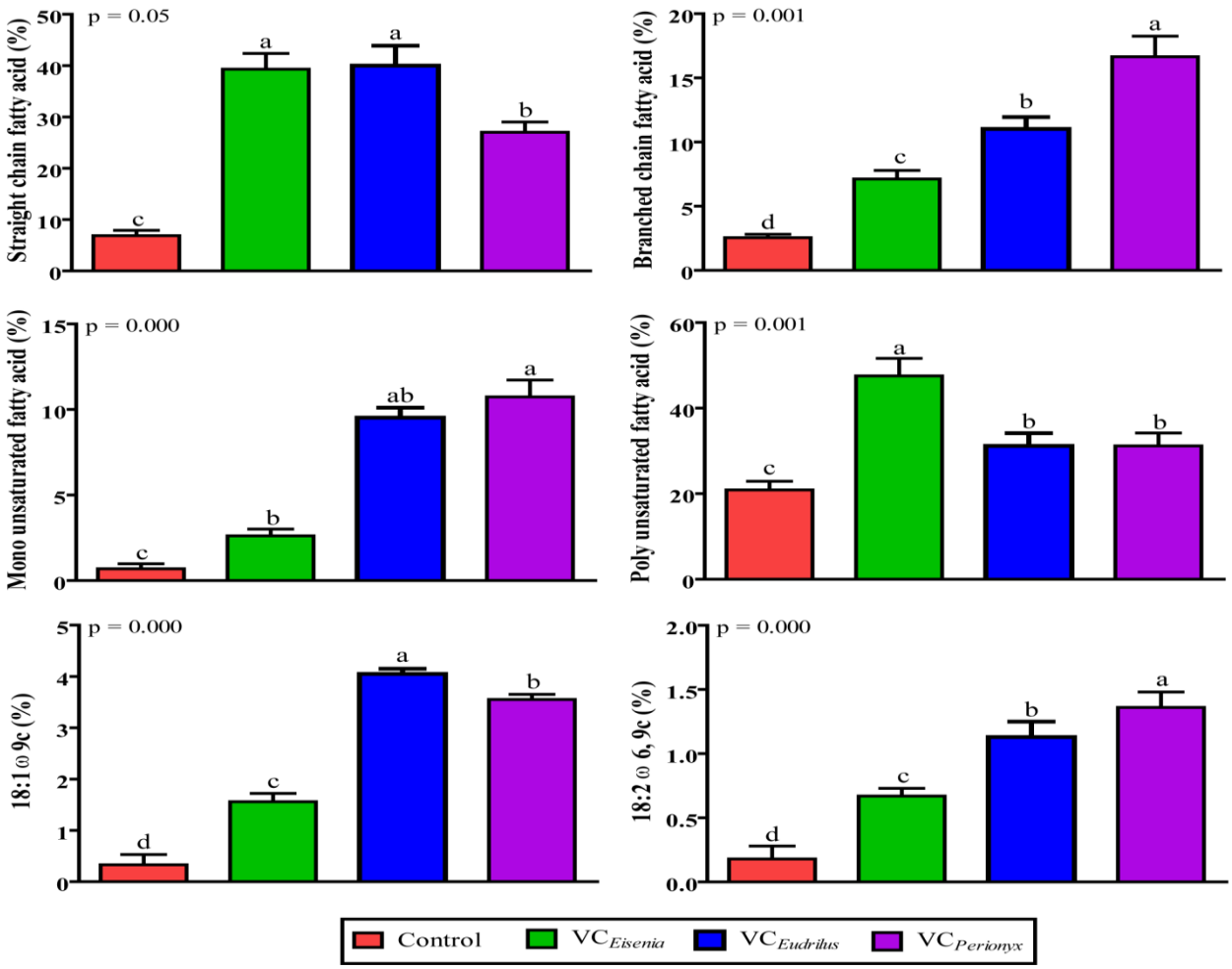


Fig. 5 Percentage composition of different types of fatty acids in the vermicomposted and composted feedstocks detected through Phospholipid fatty acid (PLFA) analysis.



3.4 Histological studies, stress indicators and DNA degradation studies in MSW exposed environment

Effects of MSW on earthworm health:

The effects of MSW on cellular structure of earthworms were evaluated based on histological assay following the procedure of Sharma and Satyanarayan (2011). In addition, the activity of various stress indicators was also studied on the MSW treated worms.

Histological analysis and stress enzyme activity:

Compact associations of epithelial cell lining of lumen and chloragogenous tissue (CT) have been observed in control earthworm. The loss of structural integrity in CT and its detachment from the epithelial lining of lumen was noted in treated earthworm specimens. **S**tress enzyme assay was performed to find out the mechanism by which earthworms respond to the heavy metal stress. Earthworms subjected to different feed stocks were sacrificed for the analysis

(Table 1). The table depicts that increase in solid waste proportion in feedstock elevates oxidative stress in earthworms.

Fig 6: Histological study of the earthworm

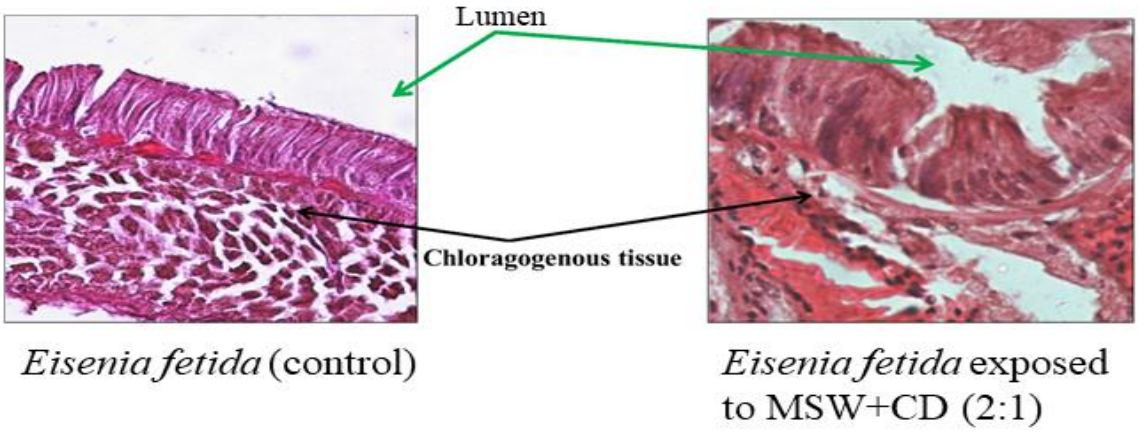


Table 1: Total Protein and Stress Enzyme assay in treated Earthworm

Feedstock	Catalase activity/mg protein	SOD activity/mg protein	Lipid peroxidase activity (µM $gmin^{-1}$)
V1	**70.42**	**2.86**	**0.02**
V2	**98.59**	**11.03**	**15.79**
V3	**161.97**	**4.28**	**15.74**
V4	**133.80**	**5.32**	**14.22**

Where V1=CD, V2- Only solid waste (SW), V3- SW: CD (1:1), V4- SW+CD(2:1)

3.5 Identification of non-metallothionein metal binding proteins in earthworm gut

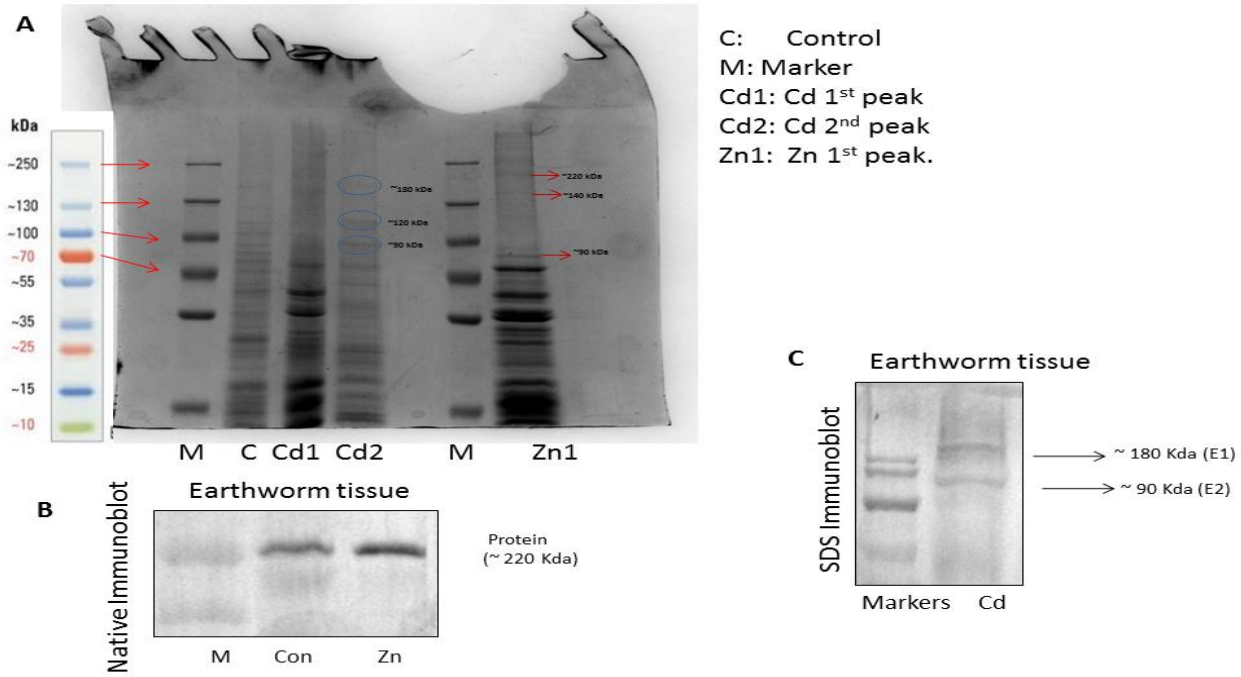
Assessment of crude protein contents (table 2) in earthworm body reveled that Protein concentration was higher in the treated samples in comparison to control samples. Further in all the sections protein concentration was higher in Zn treated earthworms than Cd treated ones. The result is in synchrony with the fluorescence emission array. Among the three sections anterior, mid and posterior the mid-section showed highest fluorescence emission in both Cd and Zn followed by anterior and posterior. Similar kind of florescence emission pattern in case of both Cd and Zn depicts similar mechanism of accumulation of both the metals.

Table 2 Crude protein levels in different body sections of *E. fetida*

	Earthworm (Anterior portion) (µg/ml)	Earthworm (Mid portion) (µg/ml)	Earthworm (Posterior portion) (µg/ml)
Control	**13.85**	**10.78**	**7.75**
Treated (Cd)	**16.54**	**12.50**	**11.44**
Treated (Zn)	**17.31**	**14.36**	**12.42**

Previous studies showed that earthworms neutralize toxic metals through a chelation pathway mediated by a small (~ 13 kDa) cysteine rich protein, metallothionein (MT). However, MT expression and the extent of metal accumulation often do not correlate, although the reason behind such incoherence remains unclear. Our work demonstrates that expression of a few metal induced, high-molecular weight proteins in earthworm (*Eisenia fetida*) is responsible for such incongruity. Applying selective protein isolation techniques in earthworms exposed to fluorescence tagged cadmium and zinc compounds; we were able to detect three (~90 kDa, 120 kDa, and ~180 kDa) such cadmium induced and three zinc induced (~220 kDa, 140 kDa, and 90 kDa) proteins, respectively through gel electrophoresis (Fig. 7A). Moreover, the 180 kDa and 90 kDa of Cd induced and 220 kDa of Zn induced proteins were again identified through immunoblotting (Fig. 7 B & C).

Fig. 7. Gel electrophoresis and immunoblots assays for metal induced proteins in earthworm gut



3.6 Effect of vermicomposted solid waste on maize production

3.6.1 Physico-chemical characteristics of field soil, vermicompost and biochar

The physico-chemical properties of the soil of the experimental field, MSW-vermicompost (VCM) and biochar (BC) are detailed in Table 3. The soil was moderately acidic (pH 5.63) with low water retention capacity and bulk density. On the other hand, the pH of VCM was near neutral, while the BC was highly alkaline in nature. The water retention capacity (WRC) of VCM and BC was remarkably higher than the soil, while WRC of biochar was significantly highest. BD of the three constituents (Soil, VCM and BC) was in the order soil> VCM>BC [p(sub)<0.01; LSD= 5.95]. The total nitrogen content of the soil was significantly lower as compared to BC and VCM [p(sub)<0.01; LSD= 5.95]. Whereas, the TN contents of the VCM and BC was almost similar; while availability of P and K was slightly higher in BC than VCM. The TOC content was significantly high in BC followed by VCM and soil. Microbial biomass carbon and respiration were significantly higher in VCM than BC and soil. Such high microbial activity in vermicompost is largely attributed to the contributions of earthworm intestinal microflora. The occurrence of essential and non-essential metals in soil, VCM and BC were well below the critical limit for agricultural soils. However, among the three constituents

concentration of Cd, Cr, Pb, and Fe was significantly higher in VCM as compared to others [Table 7.4, p(sub)<0.01; LSD= 5.95].

Table 3: Physicochemical properties of the experimental soil, MSW-vermicompost (VCM) and bio-char (BC) used. Values represented in mean ± standard deviation

Parameters	Soil	VCM	BC
WHC (%)	38.0±2.0	111.22± 6	129 ± 9
BD	1.22±0.03	0.46±0.03	0.24±0.02
pH	5.63±0.32	5.98±0.04	10.76±0.1
TKN (%)	0.15±0.09	0.95±0.05	0.92±0.07
Av P(mg kg^{-1})	35.21±4.64	137±25	169±79.0
Av K (mg kg^{-1})	88.36±16.70	256.7±89	281.12±107
TOC (%)	1.28±0.16	5.07±0.4	24.17±2.2
Microbial biomass C (µg g^{-1})	232.12±20.21	1468.7±55.2	522.11±45.3
Microbial respiration (µg g^{-1})	0.37±0.02	0.98±0.05	0.42±0.03
Av. Cd (mg kg^{-1})	0.003±0.001	0.032±0.002	0.015± 0.001
Av. Cr (mg kg^{-1})	0.02± 0.001	0.49±0.04	0.22±0.03
Av. Pb (mg kg^{-1})	0.02±0.002	0.39±0.04	0.17±0.02
Av. Zn (mg kg^{-1})	0.36±0.04	51.64±4.36	63.12±3.56
Av. Cu (mg kg^{-1})	0.02± 0.002	3.95±0.35	8.34±0.02
Av. Fe (mg kg^{-1})	1.32±0.1	526.94±43.2	456.32± 42.31
p(substance)	<0.01		
$LSD_{substance}$	5.95		

3.6.2 Effect of different treatments on water retention capacity (%), bulk density (g cc^{-1}) and pH of soil under cultivation

The data on changes in water retention capacity (WRC), bulk density and pH are represented in Table 4, 5 and 6 respectively. The WRC of the soil varied significantly under different treatment combinations [p(treatment) < 0.01; $LSD_{treatment}$= 2.27] and also within year [p(year) <0.01; LSD_{year}= 1.51]. After two years of cultivation significant improvement in WRC was recorded in T_7 and T_8 followed by T_3, T_6 and T_4. The result indicates that incorporation of organic fertilizers specially VCM improved aggregate stability of the soil which in turn enhanced WRC of the soil.

Table 4: Changes in the water retention capacity (%) of soil treated with different nutrient scheme under *Zea maize* cultivation. Values represented in mean ± standard deviation

		Water retention capacity (%)				
		Year 1			Year 2	
Treatment	NPK*, VC ton/ha, BC ton/ha	15 DAS	117 DAS	Fallow	15 DAS	117 DAS
T_1	0.5, 0, 0	42.01 ± 7.01	55.13 ± 4.02	41.21± 3.97	45.15±0.71	65.25±0.54
T_2	0.5, 0, 3	44.04 ± 14.00	50.22 ± 5.03	48.23±4.11	53.93±0.69	68.12±0.89
T_3	0.5, 1.5, 0	47.02 ± 11.21	51.14 ± 6.12	47.23±4.23	48.11±0.59	67.25±1.00
T_4	0.5, 1.5, 3	45.11 ± 13.22	55.15 ± 1.21	50.23±4.76	51.23±0.41	60.68±0.57
T_5	1, 0, 0	46.13 ± 6.01	46.23 ± 7.32	45.15±4.11	50.46±0.66	67.53±0.89
T_6	1, 0, 3	49.02 ± 8.03	64.75 ± 10.21	55.23±5.28	53.13±0.88	56.86±0.71
T_7	1, 1.5, 0	45.03 ±10.21	66.21 ± 6.04	50.12±4.72	52.02±0.99	75.54±0.50
T_8	1, 1.5, 3	39.04 ± 2.03	53.33 ± 6.07	52.11±4.90	45.32±0.44	68.28±0.09
T_9	0.75, 0.75, 1.5	46.11 ± 2.05	49.21 ± 8.06	44.21±4.13	45.58±0.25	49.99±0.92
	p(treatment)	<0.01				
	p(time)	<0.01				
	p (treatment*time)	<0.01				
	$LSD_{treatment}$	2.27				
	LSD_{time}	1.51				

ᵋ **Dose of Recommendation**

In our study the BD significantly reduced over time due to application of VCM and BC treatments over two years [p(tre) < 0.01, LSD_{tre}= 0.05; p(time) <0.01, LSD_{time}= 0.01]. After two years of cultivation, remarkable reduction in BD was recorded in T_7 and T_3 followed by T_6, T_4, T_2, T_9 and T_8. Whereas, such reduction in BD was insignificant in chemical fertilizered soil (T_1 and T_5). In general, organic manuring improves physical environment of soil by increases porosity of soil through the process of humification Schaeffer et al.,2015; Blanco-Canqui et al., 2007. Biochar generally reduces soil BD by 3 to 31% Adekiya et al.,2020. On the other hand, vermicompost being earthworm mediated manure is composed of porous and stable aggregates, which in turn probably facilitated the improvement of porosity in treated soil Goswami et al.,2014.

Table 5: Changes in the bulk density (g cc^{-1}) of soil treated with different nutrient scheme under *Zea maize* cultivation. Values represented in mean ± standard deviation

		Bulk density				
		Year 1			Year 2	
Treatment	NPK*, VC ton/ha, BC ton/ha	15 DAS	117 DAS	Fallow	15 DAS	117 DAS
T_1	0.5, 0, 0	1.25±0.02	1.15±0.07	1.18±0.01	1.07±0.02	0.76±0.02
T_2	0.5, 0, 3	1.23±0.02	1.17±0.10	1.20±0.02	0.98±0.01	0.74±0.01
T_3	0.5, 1.5, 0	1.25±0.02	1.20±0.06	1.21±0.02	1.12±0.00	0.79±0.01
T_4	0.5, 1.5, 3	1.24±0.03	1.15±0.02	1.18±0.01	1.10±0.01	0.79±0.02
T_5	1, 0, 0	1.25±0.05	1.12±0.10	1.14±0.01	1.06±0.02	0.76±0.01
T_6	1, 0, 3	1.25±0.01	1.10±0.04	1.13±0.02	1.05±0.02	0.77±0.01
T_7	1, 1.5, 0	1.27±0.01	1.12±0.08	1.15±0.01	1.09±0.03	0.80±0.01
T_8	1, 1.5, 3	1.26±0.02	1.13±0.02	1.17±0.01	1.12±0.03	0.75±0.05
T_9	0.75, 0.75, 1.5	1.25±0.03	1.11±0.04	1.05±0.02	1.08±0.02	0.74±0.01
	p(treatment)	<0.01				
	p(time)	<0.01				
	p (treatment*time)	NS				
	$LSD_{treatment}$	0.05				
	LSD_{time}	0.01				

‘ **Dose of Recommendation**

Soil pH significantly fluctuated under different treatments over time [p(tre) < 0.01; LSD_{tre} = 0.08]. The soil pH increased as compared to the initial value at the earlier stage of crop cultivation but reduced during the harvest in time of first crop. At the end of 2nd year soil pH was highest under T_2 followed by T_3 and T_4 [Table7.7, p(tre) <0.01; LSD_{tre}=0.08]. These results indicate that substitution of inorganic fertilization with BC and VCM may help in addressing the problem of soil acidification in long run Thu et al., 2015.

Table 6: Changes in pH of soil treated with different nutrient scheme under *Zea maize* cultivation. Values represented in mean ± standard deviation

		pH				
		Year 1			Year 2	
Treatment	NPK*, VC ton/ha, BC ton/ha	15 DAS	117 DAS	Fallow	15 DAS	117 DAS
T_1	0.5, 0, 0	6.00±0.12	4.56±0.08	4.8±0.01	5.6±0.24	5.53±0.06
T_2	0.5, 0, 3	5.98±0.11	5.58±0.30	6.22±0.04	5.49±0.14	5.73±0.25
T_3	0.5, 1.5, 0	5.95±0.10	4.46±0.06	5.23±0.02	5.74±0.42	5.63±0.29
T_4	0.5, 1.5, 3	5.96±0.09	5.59±0.24	5.82±0.05	5.94±0.16	5.63±0.25
T_5	1, 0, 0	5.82±0.05	4.53±0.21	5.12±0.04	5.74±0.09	5.21±0.02
T_6	1, 0, 3	5.79±0.05	5.50±0.18	5.34±0.03	5.93±0.14	5.37±0.21
T_7	1, 1.5, 0	5.79±0.06	4.38±0.12	5.44±0.04	5.96±0.48	5.37±0.12
T_8	1, 1.5, 3	5.75±0.04	5.47±0.26	5.24±0.03	5.02±0.39	5.40±0.10
T_9	0.75, 0.75, 1.5	5.68±0.32	4.43±0.12	5.13±0.02	5.61±0.25	5.47±0.32
	p(treatment)	<0.01				
	p(time)	<0.01				
	p (treatment*time)	<0.01				
	$LSD_{treatment}$	0.08				
	LSD_{time}	0.05				

‘ Dose of Recommendation

3.6.3 Changes in Soil organic carbon (%), Total Kjeldahl nitrogen (%), Available phosphorus (mg kg^{-1}), and Available potassium (mg kg^{-1}) of soil under different treatments

The soil was inherently rich in SOC (Table 7). However, depending on treatment combinations SOC also significantly varied with time [p(time)<0.01, P(tre*time) <0.01, LSDtime= 0.05]. The SOC reduced during the fallow season irrespective of treatments; which substantially regained during the early vegetative growth stage of the second year of crop. After two years of cultivation the highest gain in SOC was observed in T_7 (100% Recommended NPK + VCM 1.5 t ha^{-1}) (2.58) fold followed by T_8 (100% Recommended NPK+ VCM1.5 t ha^{-1}+ BC3 t ha^{-1})

(2.56) fold. At the end of the second year, the SOC under different treatments were in the order T_7> T_8>T_6> T_5> T_4>T_3> T_9> T_2> T_1 (Table 7). Overall, the VCM application resulted in greater SOC build up in soil as compared to BC. This may be due to the release of several organic acid carbons; humic acid carbon (HAC) and fulvic acid carbon (FAC) from the vermicompost Das et al., 2019 ; Sahariah et al., 2020.

Table 7: Changes in the Soil organic carbon (%) of soil treated with different nutrient scheme under *Zea maize* cultivation. Values represented in mean ± standard deviation

		Soil organic carbon (%)				
		Year 1			Year 2	
Treatment	NPK*, VC ton/ha, BC ton/ha	15 DAS	117 DAS	Fallow	15 DAS	117 DAS
T_1	0.5, 0, 0	1.31±0.12	1.50±0.24	1.21±0.12	1.84±0.15	2.46±0.22
T_2	0.5, 0, 3	1.44±0.13	1.33±0.18	1.15±0.13	2.17±0.14	2.52±0.25
T_3	0.5, 1.5, 0	1.47±0.17	1.91±0.16	1.34±0.11	2.24±0.14	2.69±0.22
T_4	0.5, 1.5, 3	1.43±0.16	1.56±0.10	1.18±0.12	2.57±0.25	2.86±0.32
T_5	1, 0, 0	1.53±0.20	1.79±0.15	1.23±0.12	2.70±0.24	3.06±0.26
T_6	1, 0, 3	1.66±0.21	1.74±0.22	1.34±0.11	2.79±0.19	3.21±0.23
T_7	1, 1.5, 0	1.54±0.11	1.57±0.09	1.37±0.13	2.77±0.24	3.81±0.29
T_8	1, 1.5, 3	1.51±0.17	1.64±0.14	1.38±0.14	2.84±0.24	3.63±0.30
T_9	0.75, 0.75, 1.5	1.67±0.16	1.75±0.13	1.26±0.12	1.98±0.17	2.57±0.24
	p(treatment)	<0.01				
	p(time)	<0.01				
	p (treatment*time)	<0.01				
	$LSD_{treatment}$	0.08				
	LSD_{time}	0.05				

*** Dose of Recommendation**

The data on changes in total Kjeldahl nitrogen (TKN), available phosphorus (Av. P) and available potassium (Av. K) are represented in Table 8 and 9 respectively. The availability of nitrogen in soil is a major determinant of crop production.

Table 8: Changes in the soil Total Kjeldahl Nitrogen (%) treated with different nutrient scheme under *Zea maize* cultivation. Values represented in mean ± standard deviation

Total Kjeldahl nitrogen (%)						
		Year 1			Year 2	
Treatment	NPK*, VC ton/ha, BC ton/ha	15 DAS	117 DAS	Fallow	15 DAS	117 DAS
T_1	0.5, 0, 0	0.17±0.03	0.09±0.02	0.08±0.01	0.40±0.02	0.21±0.03
T_2	0.5, 0, 3	0.18±0.03	0.12±0.03	0.10±0.02	0.44±0.04	0.28±0.03
T_3	0.5, 1.5, 0	0.32±0.03	0.15±0.02	0.12±0.02	0.78±0.06	0.38±0.02
T_4	0.5, 1.5, 3	0.25±0.07	0.14±0.01	0.11±0.01	0.79±0.11	0.43±0.03
T_5	1, 0, 0	0.22±0.11	0.11±0.02	0.10±0.01	0.61±0.09	0.34±0.03
T_6	1, 0, 3	0.26±0.10	0.15±0.05	0.13±0.02	0.65±0.02	0.39±0.02
T_7	1, 1.5, 0	0.26±0.07	0.17±0.07	0.13±0.02	0.80±0.04	0.41±0.02
T_8	1, 1.5, 3	0.28±0.09	0.19±0.05	0.12±0.01	0.82±0.06	0.47±0.04
T_9	0.75, 0.75, 1.5	0.28±0.09	0.12±0.01	0.10±0.01	0.79±0.06	0.30±0.04
	p(treatment)	<0.01				
	p(time)	<0.01				
	p (treatment*time)	<0.01				
	$LSD_{treatment}$	0.02				
	LSD_{time}	0.01				

‘ **Dose of Recommendation**

The initial value of TNK in soil was substantially low (Table 8). Overall, a significant increment in TKN in soil was observed at the initial stage of crop growth during the first year irrespective of treatments; which sharply reduced after harvesting of the first crop and the trend of reduction in TKN was continued during the fallow season. This may be due to removal of plant biomass after crop maturation. Maize is known as an exhaustive crop which removes nutrient from soil in substantial amount Pooniya et al., 2013. However, during the early vegetative growth stage of the second crop sharp increment in nitrogen content in soil was recorded under T_8 followed by T_7, T_9, T_4 and T_3 [$p(tre)<0.01$; $LSD_{tre}=0.02$]. The two-way ANOVA also revealed that the temporal variation of soil N under various treatments was significant as well as the (treatment* time) interaction was also significant. However, the TKN

in soil after harvesting of the second year crop was in the order T_8> T_7> T_4> T_6> T_3 >T_5 T_9>T_2>T_1. Overall, all the results imply that combined application of VCM and BC could not only restore the N fertility in soil but also enhanced N content despite of cultivation of an exhaustive crop like maize.

The soil was inherently low in P availability, which is largely attributed to the acidic soil reaction (Table 9). Such characteristics of alluvial soil of Assam has been reported earlier Saikia et al., 2015. However, the inherent K level was moderate in the soil Tandon (1995). Overall availability of both P and K in soil considerably increased after two years of maize cultivation with VCM and BC based treatments; although sharp temporal change in P and K availability was evidenced as found in case of nitrogen. Maize being an exhaustive crop removes the macro and micro nutrients from soil Pooniya et al., 2013. However, it was interesting to know that the fold raise in P content from basic soil after two years was highest in T_8 (2.58 fold) followed by T_7 (2.34 fold) and T_4 (2.29 fold). At the early vegetative growth of the crop K in soil was highest under T_8 followed by T_6 and T_7 in the 1st year; while after two years of cultivation K availability in soil under various treatments was in the order T_8> T_4> T_7>T_9>T_3> T_6>T_5> T_2>T_1 [p(tre)=<0.01; LSD=14.23]. Moreover, the highest fold-rise in K availability in soil as compared to the initial value was under T_8 (3.28 fold). Never the less, the remarkable initial gain in K availability in soil during the first year gradually reduced over time probably due to crop removal and leaching loss Sahariah et al., 2020. Interestingly, the results indicate that conjoint application of VCM and BC considerably facilitated macro nutrient (N, P, and K) restoration in soil under mono-cropping of maize.

Table 9: Changes in the soil Available phosphorus (mg kg^{-1}) and Available potassium (mg kg^{-1}) treated with different nutrient

		Available phosphorus(mg kg^{-1})					Available potassium(mg kg^{-1})				
		Year 1			Year 2		Year 1			Year 2	
Treatment	NPK*, VC ton/ha, BC ton/ha	15 DAS	117 DAS	Fallow	15 DAS	117 DAS	15 DAS	117 DAS	Fallow	15 DAS	117 DAS
T_1	0.5, 0, 0	62.84±6.82	43.93±3.5	32.11±2.80	50.80±4.60	45.00±2.76	334.49±36.65	125.67±12.55	97.23± 6.54	454.97± 40.40	21.40±10.53
T_2	0.5, 0, 3	71.52±4.08	60.26±5.65	40.23±3.62	64.83±6.40	48.97±3.93	381.50±35.23	127.49±13.00	100.12± 7.23	515.97±50.81	28.90±11.61
T_3	0.5, 1.5, 0	63.03±5.15	56.70±4.95	43.12±3.94	67.00±7.09	52.01±6.39	369.41±36.62	158.98±14.84	112.23± 9.31	571.43±53.43	23.40±20.46
T_4	0.5, 1.5, 3	69.62±8.00	59.77±4.71	42.24±3.70	109.55±9.49	82.76±6.96	421.73±33.85	147.11±17.29	108.56± 7.21	610.43±60.75	76.97±24.15
T_5	1, 0, 0	67.56±7.93	48.73±3.53	38.14±3.22	56.51±3.87	55.34±4.37	371.42±42.54	155.04±16.21	107.78± 8.23	469.40±43.62	65.43±15.51
T_6	1, 0, 3	70.41±4.29	69.67±7.22	43.74±3.81	85.65±9.75	73.61±6.48	490.39±42.39	131.97±11.51	111.24±10.11	528.73±50.96	95.63±18.60
T_7	1, 1.5, 0	75.89±7.32	64.26±7.38	45.24±4.13	121.36±10.44	82.44±6.99	473.26±31.13	138.06±15.80	119.54± 9.23	600.43±60.90	45.30±20.61
T_8	1, 1.5, 3	106.33±6.05	72.40±7.25	47.14±3.72	127.73±11.18	91.00±10.98	514.52±48.18	154.89±11.28	121.52±10.12	671.97±63.67	90.57±23.51
T_9	0.75, 0.75, 1.5	81.21±7.64	61.91±6.26	42.56±4.11	76.82±8.34	58.91±4.77	378.60±36.30	184.90±18.77	109.26± 9.31	484.13±47.45	30.37±20.91
	p(treatment)	<0.01					<0.01				
	p(time)	<0.01					<0.01				
	) (treatment*time)	<0.01					<0.01				
	$LSD_{treatment}$	2.77					14.23				
	LSD_{time}	1.85					9.49				

ˢ Dose of Recommendation

scheme under *Zea maize* cultivation. Values represented in mean ± standard deviation

3.6.4 Changes in Microbial biomass carbon (µg g^{-1}) and microbial respiration (µg g^{-1}) of soil under different treatment

Microbial biomass carbon and respiration in the soil was moderate at the time of commencement of the experiment (Table 10). Both MBC and respiration significantly fluctuated over time depending on the treatment combination [MBC: p(tre)<0.01, p(time)= <0.01, p(tre*time) =<0.01, $LSD_{treatment}$=49.18; MR= p(tre)<0.01, p(time)= <0.01, p(tre*time) =<0.01 $LSD_{treatment}$=0.135]. After two years of cultivation MBC increment was significantly greater in T_7 treated plots followed by T_8, T_4 and T_3. This is interesting because all these treatments were constituted either with VCM or VCM-BC combinations. On the other hand, sole application of BC (T_2 and T_8) and synthetic fertilizer (T_1 and T_5) substantially retarded MBC in soil. However, respiration was significantly higher in T_8 followed by T_7 and T_9. In fact, biochar application resulted in augmentation of soil respiration in the present experiment (T_8, T_6, T_9). Moreover, soil respiration was considerably lower in plots treated with 50% reduce chemical fertilizer (T_1-T_4). Therefore, synthetic fertilization and biochar application have resulted in augmentation in microbial respiration which reflects that the activity of metabolically dormant (Autochthonous microflora) was facilitated in these cases Tripathi et al., 2014. In fact, activation of autochthonous microbial population indicates a stressful unfavorable condition for plant biomass yield Rusinowski et al., 2019.

Table 10: Changes in the Microbial biomass carbon (µg g^{-1}) and respiration (µg g^{-1}) of soil treated with different nutrient scheme under *Zea maize* cultivation. Values represented in mean ± standard deviation

		Microbial biomass carbon (µg g^{-1})					Microbial respiration (µg g^{-1})				
		Year 1			Year 2		Year 1			Year 2	
Treatment	NPK*, VC ton/ha, BC ton/ha	15 DAS	117 DAS	Fallow	15 DAS	117 DAS	15 DAS	117 DAS	Fallow	15 DAS	117 DAS
T_1	0.5, 0, 0	732.12±40.70	1302.66±59.38	642.22±50.23	885.45±83.85	900.34± 61.68	1.58±0.16	1.65±0.06	1.10±0.11	2.99±0.17	3.22±0.33
T_2	0.5, 0, 3	1297.05±70.57	1743.37±47.43	745.62±67.21	1300.61±101.85	1501.83± 81.55	1.68±0.12	1.71±0.24	1.16±0.20	3.31±0.32	4.28±0.26
T_3	0.5, 1.5, 0	1471.73±65.88	2012.33±112.32	784.22±68.32	1557.85±161.01	2197.00±201.98	2.36±0.39	2.67±0.18	1.20±0.13	3.41±0.23	4.65±0.47
T_4	0.5, 1.5, 3	1519.62±102.61	2049.83±156.88	820.21±73.24	1427.85±128.32	2331.01±164.04	3.28±0.27	3.56±0.18	1.22±0.11	3.79±0.34	4.87±0.36
T_5	1, 0, 0	1102.45±43.13	1159.56±104.81	689.21±57.21	1097.94±76.9	1149.79±105.96	1.37±0.36	2.33±0.20	1.13±0.13	4.39±0.33	5.30±0.49
T_6	1, 0, 3	1395.05±76.77	1474.96±110.75	776.23±61.22	1367.29±91.05	1467.89±142.70	1.51±0.19	2.82±0.17	1.18±0.21	4.76±0.37	6.26±0.66
T_7	1, 1.5, 0	2190.65±112.05	2508.43±150.89	800.31±64.47	1643.58±125.29	2779.57±269.51	2.67±0.33	2.75±0.15	1.22±0.22	4.97±0.36	6.95±0.66
T_8	1, 1.5, 3	2460.67±103.01	2605.49±69.59	856.23±69.46	1831.43±138.67	2636.46±242.00	4.03±0.26	3.44±0.27	1.25±0.25	5.28±0.57	7.83±0.33
T_9	0.75, 0.75, 1.5	853.30±56.37	1072.82±35.37	769.23±59.23	907.04±90.59	1240.02±117.22	1.65±0.18	1.74±0.17	1.22±0.16	4.61±0.42	6.22±0.26
	p(treatment)	<0.01					<0.01				
	p(time)	<0.01					<0.01				
	) (treatment*time)	<0.01					<0.01				
	$LSD_{treatment}$	49.18					0.135				
	LSD_{time}	32.79					0.09				

ᵎ Dose of Recommendation

3.6.5 Data on changes in availability of essential and non-essential metals in soil

The concentrations of all the metals significantly reduced in soil over time irrespective of treatment combination (Fig.8). However, treatment wise variation in regard to metal concentration in soil was not significant for non-essential metals (Cd, Cr, Pb) and also for essential micronutrients like (Fe and Cu). Never the less the beneficial impact of vermicompost and biochar application could be significantly appreciated in regard to Zn reduction under T_8, T_6, T_9 and T_5 treatments. Interestingly, Zn concentration was lowest after two years of maize cultivation in BC+VCM treated soil. Such positive impact of vermicompost application has been reported by Goswami et al., 2017 in fact vermicompost application triggers the transformation of bioavailable metal forms to insoluble forms via formation of organometallic complexes.

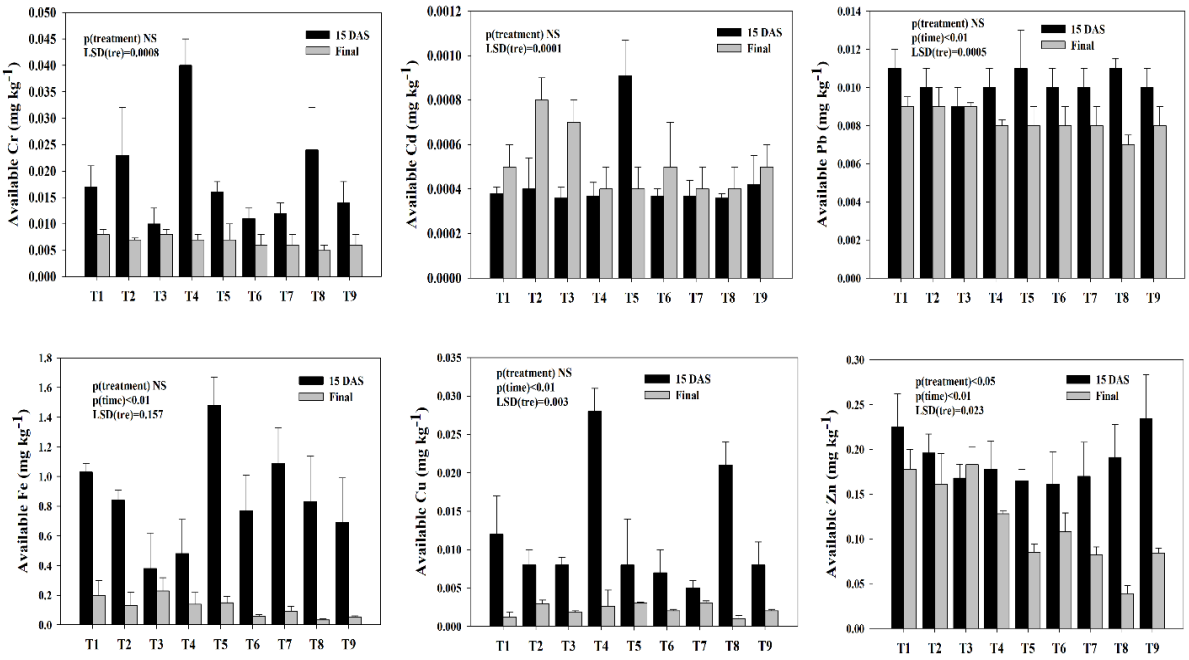


Fig. 8: Changes in availability of essential and non-essential metals in soil at 15DAS (first year) and Final (harvest 2nd year)

3.6.6 Impact of MSW-Vermicompost (VCM) and Biochar (BC) on crop yield

The data on maize yield under various treatments are presented in (Table 11). In the 1st year the cob yield was significantly high in T_6 treated plots followed by T_6 and T_8. Interestingly, maize

yield significantly increased in the 2nd year as compared to 1st year under all the treatments except T_6 (NPK100%+BC) and T_1 (NPK 50% only) in fact there was about 11% yield reduction in T_6 treated plots in the 2nd year; there was 12.8% increase in maize production under T_8 (i.e., combined application of biochar and vermicompost). In the 2nd year the cob yield under various treatments were of the order $T_8>T_7>T_9=T_4\geq T_6=T_3=T_5\geq T_2>T_1$ [$p(tre)<0.01$; $LSD_{tre}=3.79$]. The results strongly indicate that use of biochar as sole organic input may suppress crop productivity in the long run. As such, biochar application may greatly affect nutrient bioavailability and induce soil salinity, which in turn lead to substantial reduction in crop productivity Brtnicky et al. 2021. On the other hand, sole application of VC and BC-VCM combination resulted in significant yield increment in the present experiment. In fact, 50% reduction in inorganic fertilization was greatly compensated by BC-VCM substitution (i.e., T_4).

Table 7.14: Effect of different treatments on cob yield of *Zea maize* plants grown under different nutrient scheme. Values represented in mean ± standard deviation

Cob Yield			
		Year 1	**Year 2**
Treatment	**NPK*, VC ton/ha, BC ton/ha**	**Harvest (quintal h^{-1})**	**Harvest (quintal h^{-1})**
T_1	**0.5, 0, 0**	72.22±6.00	70.7± 6.22
T_2	**0.5, 0, 3**	51.85±8.49	74.27±7.1
T_3	**0.5, 1.5, 0**	72.22±5.00	77.4±7.2
T_4	**0.5, 1.5, 3**	77.78±8.00	81.98±7.4
T_5	**1, 0, 0**	61.11±5.00	76.55±6.7
T_6	**1, 0, 3**	89.85±4.19	79.64±6.8
T_7	**1, 1.5, 0**	86.67±2.94	88.06±8.1
T_8	**1, 1.5, 3**	84.33±6.00	95.15±8.8
T_9	**0.75, 0.75, 1.5**	77.04±1.28	83.24±7.9
	p(treatment)	<0.01	
	p(time)	<0.01	
	p (treatment*time)	<0.01	
	$LSD_{treatment}$	3.79	

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Submitted:

FORM-L
UTILISATION CERTIFICATE

COUNCIL OF SCIENTIFIC AND INDUSTRIAL RESEARCH
Human Resource Development Group
CSIR Complex, Library Avenue, Pusa, New Delhi – 110012

CSIR-HRDG Scheme No. 38/1445/17/EMR-II

S.No.	Particulars	Letter No. /Bank Transaction ID Nos. & Date	Amount
1	Grants received from CSIR during the year	38(1445)/17 dt. 18.2.2020	Rs. 6,69,867.00
2	Unspent balance of previous year	-DO-	Rs. 1,10,661.00
3	Interest earned/accrued on CSIR grant	N/A	Rs. 5886.00
		Total	Rs. 7,86,414.00

1. Certified that out of Rs. 6,69,867.00 (Rupees SIX LAKH SIXTY NINE THOUSAND EIGHT HUNDRED SIXTY SEVEN) only of grant-in-aid released by Extramural Research (EMR) Division of HRDG (CSIR) vide letter No./Bank Transaction ID Nos. 3(1445)/17 dated 18.2.2020 as given in the margin during the year 2019-2020 and Rs. 5886.00 only earned/accrued as interest from bank on grants released by CSIR and Rs. 1,10,661.00 only on account of unspent balance of the previous year, a sum of Rs. 4,80,528.00 only has been utilized for the purpose for which it was sanctioned and that the balance of Rs. 3,05,886.00 remaining unutilized at the end of the year because the fund was actually transferred during the succeeding financial year (i.e., April 2020 to March 2021), hence, cannot be adjusted towards the grant-in-aid payable during the next year.

2. Certified that I have satisfied myself that the conditions on which the grants-in-aid was sanctioned have been duly fulfilled/are being fulfilled and that I have exercised the following checks to see that the money was actually utilized for the purpose for which it was sanctioned. The detail expenditure incurred during the year is shown in the enclosed "Statement of Accounts (Receipt & Payment)".

(Kinds of checks exercised)

1. Vouchers and Statement of Accounts
2. Grant-in-Aid

3. Expenditure Register
4. Bank statements for accrual interest
5.

Signature of Authorised Officer
with Date& Seal

Finance Officer
Tezpur University

Countersigned by Registrar/Dean/Director
Of the institute with Date & Seal

Registrar
Tezpur University

The Utilization certificate and statement should be signed by Head of the Finance & Accounts and countersigned by Registrar/Dean/Director of the University/Institute.

Consolidated Statement of Accounts

(From the date of commencement : 1st October 2017 , till 31st March 2021)

Scheme Number: 38(1445)/11/EMR / -II date 25/05/2017

Title of the Research Scheme : **"Prospect of a novel protein in earthworm gut to minimize heavy metal risk in vermicompost for sustainable crop production"**

Name of the Investigator-in-Charge : Dr. S.S. Bhattacharya
Date of Commencement : 1st October 2017 Date of Termination: 31st march 2021

Receipts (Particulars of grants received) (in Rs.)								Payments (Particulars of grants spent) (in Rs.)						
Period (ending 31March)	Cheque No., date & Amount	Stipend period ending 31 March, 2020	Contingency	Scientist Allowance (for Emeritus Scientist Scheme only)	Equipment Grant	HRA + MA	Total	Stipend	Contingency	Scientist Allowance (for Emeritus Scientist Scheme only)	Equipment Grant	HRA + MA	Total	Balance
1st Oct, 2017 to 31st March 2021	Online funds transfer **2306481** (775000+624014+ 237600+669867)	1068545	723316	Nil	416664	97956	2306481	1068545	721788	Nil	416664	97956	2304953	1528

Signature of Registrar with Stamp

Registrar
Tezpur University

Signature of Finance Officer with Stamp

Finance Officer
Tezpur University

Signature of PI with Stamp

22/9/21

P. I.
&
Assistant Professor
Dept of Environmental Science
Tezpur University