Annexure A: Final Technical Report

1. Introduction

Solid waste generation is a continually growing problem at global, regional and local levels. Improper disposal of these wastes pollutes all the vital components of the living environment. Biodegradable waste such as vegetable waste faces serious environmental challenges like greenhouse gas emission, odour and leachate formation. Vegetable waste comprises major portion of Municipal Solid Waste (MSW) and India is known to be the second largest producer of vegetable wastes. Open dumping of such garbage facilitates the breeding for the disease vectors, at the same time creates the issues of environmental pollution. In developing countries, the municipal solid waste generation is increasing at an unprecedented rate due to unavailability of suitable facilities for treating and disposing large amount of solid waste (Kumar,2011). Therefore, there is a crucial need of effective solid waste management.This problem of waste disposal could be minimised enormously by recycling these wastes. Vermicomposting is one of those effective options which can transform varied kind of solid wastes into valuable organic fertilizer.

Under these perspectives, in the first year of the project, we adopted vermicomposting technology which showed considerable stabilization of urban solid waste (USW) collected from Tezpur town-ship by deploying two earthworm species viz. *Eisenia fetida* and *Metaphire posthuma*. Positive changes in various compost quality attributes, such as porosity, water holding capacity, pH, total N, available P, compost respiration, and total bacterial count were observed. Furthermore, we have initiated microbial analysis of bio-composted samples and subsequently few microbial communities have been isolated.

In the second year of the project, study was carried out using the vegetable waste (VW), rice straw (RS) and cow dung (CD). Urine free cowdung samples were used to expedite the bioconversion process. Epigeic earthworm species viz. *Eisenia fetida* (E), and *Perionyx excavates* (P) respectively were collected. Positive changes in pH, total organic carbon (TOC) and fractions of C like Fulvic Acid Carbon (FAC), Humic Acid Carbon (HAC), degree of humification, enzyme activity and microbial growth were observed. Significant increment in availability of N,P,K were observed.

In this project, we are specially aiming to find out the nutrient recovery potential as well as microbial diversity resulting from mixed population of different earthworm species, which would lead us to prescribe an ideal earthworm consortium for rapid and efficient composting in Indian conditions. Therefore, In the third year of the project, another set of bioconversion experiment with vegetable waste (VW) and cow dung (CD) was carried out to find out potential of different earthworm consortia (*Eisenia fetida*, *Perionyx excavates*, *Metaphire posthuma*) to study the dynamics of nutrient availability based on different time interval and identify the best combination in biodegrading vegetable waste for best quality compost. From the study, it was known that bioconversion of vegetable waste material is highly feasible through vermitechnology employing earthworm mixtures. Concurrently, we also aim to isolate rare microorganisms from vermicompost which are able to solubilize P or fix atmospheric N efficiently. Finally, a field experiment was undertaken with the most viable mixture of organic waste obtained from the bioconversion study. Hence, the importance of integrated waste management is strongly recognized in this respect.

2. Materials and Method

2.1. Collection and characterization of experimental substrate:

Two types of wastes were collected and characterized for the study. One is Urban Solid Waste and the other is vegetable waste.Urban Solid Waste (USW) was collected from 12 points of Tezpur town. The physical properties studied are bulk density, water holding capacity (WHC), particle density, porosity etc. and the chemical properties include the pH, electrical conductivity (EC), Cation Exchange Capacity (CEC), C, Total N, available P few essential micronutrients viz. Fe, Cu, Mn and Zn (Page et al., 1982; Lindsay and Norvell, 1978). Whereas, Vegetable waste(VW) was procured from a waste disposal yard of vegetable market while rice straw(RS) and cowdung (CD) were collected from nearby area, Tezpur, Assam. Physico-chemical parameters of the samples were analysed as characterized in the previous year by following established methodologies (Page et al, 1982).

2.2. Location, earthworm species, vermi-technology and lab-scale study

2.2.1. Experimental System1 (First year yard study)

Two earthworm species viz. *Eisenia fetida* and *Metaphire posthuma* respectively were selected for the vermicomposting experiment with different ratios of Urban solid waste (USW) and cow dung (CD). Prior to the incubation, the substrates i.e. different combinations of cowdung (CD) and USW were homogenized by thorough mixing. The treatment combinations were namely Only USW, USW + CD (2:1) ,USW + CD and Only CD. One kilogram of each of the treatments was placed into burnt earthen pots of 4.5 l size and was treated with (E) and without (E_0) selected epigeic earthworm specimens (at 10 worms kg^{-1}) using three replicates for each treatment.. Such a density of earthworms has been found to be ideal for carrying out vermicomposting at this laboratory (Ghosh et al. 1999). The moisture content was maintained at 50% throughout the study period by sprinkling optimum water at 2–3 d interval. The experiment was conducted for 75 days. To evaluate the efficiency of vermicomposting a series with same treatments were maintained for simple aerobic composting. The collected samples from each treatment were taken for analysis of WHC, porosity, pH, Total N, Available P and compost respiration (Page et al., 1982).

2.2.2 Experimental System 2 (Second year yard study)

One exotic and one indigenous earthworm species viz. *Eisenia fetida* and *Perionyx excavates* respectively were collected and employed for vermicomposting. The experiment was conducted for 60 days and was allowed for incubation to occur in a shaded area with regular mixing and watering the materials for adequate aeration and proper moisture.

The treatment combinations were: T1= Vermicomposting: cow dung (CD) with *Eisenia fetida*; T2= Composting: CD T3=Vermicomposting: CD with *Perionyx excavates;*T4= Vermicomposting: CD and vegetable waste (VW) (1:1) with *Eisenia fetida*; T5= Composting: VW:CD (1:1); T6= Vermicomposting: VW:CD (1:1) with *Perionyx excavates;* T7= Vermicomposting: rice straw (RS)+CD (1:1) with *Eisenia fetida;* T8= Composting: RS+CD (1:1); T9= Vermicomposting: RS+CD (1:1) with *Perionyx excavates;*T10=Vermicomposting: VW+RS+CD (2:2:1) with *Eisenia fetida*; T11= Composting: VW+RS+CD (2:2:1); T12=Vermicomposting: VW+RS+CD (2:2:1) with *Perionyx excavates*.

The samples were drawn periodically at 30 days interval. These collected samples were taken for analysis of pH, Bulk density, Total Organic Carbon (TOC), Humic acid Carbon (HAC), Fulvic acid carbon (FAC), Degree of humification (DOH),Easily mineralizable nitrogen,

Available phosphorus, Exchangeable Potassium, Total kjeldahl nitrogen (TKN). The analysis for the above parameters were done by following standard procedures (Page et al., 1982)

2.2.3 Experimental System3 (Third Year Study)

Another set of experiment was conducted with vermicomposting and composting of vegetable waste (VW) and cowdung (CD) in 4:1 ratio in order to explore the efficiency of earthworm consortium of three earthworm species (*Eisenia fetida*, *Perionyx excavates*, and *Metaphire posthuma*). The experiment was performed in perforated earthen pots of 5 L capacity. 5kg of substrate was taken in 4 different treatment combinations with 3 replicates and incubated with earthworm specimens @ 10 worm kg^{-1} of substrate. All these pots were kept under shaded area with regular mixing of the materials for adequate aeration and supplying proper moisture. The moisture level was maintained throughout the study by periodic sprinkling of adequate quantity of water. Regular temperature measurement has been recorded. The incubation was carried out for 60 days. Samples were collected periodically at 30 days interval from each of these incubated materials and taken for analysis. Besides, earthworm study was carried out where earthworm species were also collected from each of the pots at 30 days interval in order to measure the length and body weight. The treatment combinations are as follows:

Earthworm consortium	**Ratio**
Eisenia fetida : *Perionyx excavates*	1:1
Eisenia fetida : *Metaphire posthuma*	1:1
Perionyx excavates : *Metaphire posthuma*	1:1
Eisenia fetida : *Perionyx excavates* : *Metaphire posthuma*	1:1:1

2.3 Microbial analysis of vermicomposted solid wastes and earthworm gut

First year: The composted and vermicomposted USW mixtures were analyzed for total bacterial count using nutrient agar media after Cappuccino and Sherman (2005) and all cultures were prepared in triplicate.

The total bacterial count was used as one of the vital preliminary criteria to assess earthworm compatibility with the different feed mixtures comprised of different kind of solid waste and cow dung.

Second year: The composted and vermicomposted mixtures were analysed for Nitrogen Fixing Bacteria (NFBs) and Phosphate solubilizing Bacteria (PSBs) using Burk's and Pickovskyaya media and all cultures were prepared in triplicates.

$\frac{No.of\ colonies \times dilution}{Amount\ plated}$ = No. of bacteria/ml

Third year: Adult earthworms were collected and washed with sterile water and placed on a petriplate moistened with filter paper and subjected to gut evacuation for 24 hrs. After starvation, the earthworms were disinfected with 70% ethanol, and 1g of gut content was dissected out, suspended in 10ml of doubled distilled water and homogenized with autoclaved doubled distilled water for 15 minutes in a vortex mixer. For isolation of microbes serial dilutions were made and 0.1 ml of aliquots from dilution 10^{-1} until 10^{-6} were inoculated by pour plate technique. The plates were incubated for 48hrs at 28 °C. After the completion of incubation process, the number of colonies on the plates was counted to calculate the CFU (colony forming unit)/ml. The bacterial isolates were spot inoculated by repeated streaking to maintain pure colonies. Morphologically different colonies were isolated and sub cultured for further analysis.

2.4 Screening of efficient microbes

In vitro N- fixing activity and P-solubilizing activity

The bacterial isolates were screened for N-fixing ability using N-free medium. Burk's N-free medium was prepared and sterilized. After incubation the isolates were selected on the basis of the formation of maximum number of colonies and turbidity in flasks. Among all the twelve bacterial isolates tested, five isolates showed maximum colonies and formed significantly higher rate of turbidity in the N-free liquid medium which were later selected for further molecular studies. The results were expressed as Relative dominance index (RDI). They were measured using the following formula:

$$RDI = \frac{Colony\ count\ of\ a\ particular\ strain}{Total\ colony\ count} \times Weight \qquad (1)$$

The bacterial isolates were screened for P-solubilizing activity using the selective medium for P-solubilization test. Pikovskaya's agar medium was prepared and sterilized. All the test plates were incubated for 2 days. After incubation, formation of halo zones was evaluated. The results were expressed as solubilization index (SI). They were measured using the following formula:

$$SI = \frac{Colony\ diameter + Halozone\ diameter}{Colony\ diameter} \quad (2)$$

Four bacterial strains were identified to show highest SI among all the isolates. These strains were grouped as PSB and were carried for further molecular analysis.

2.5 Genomic DNA extraction and PCR amplification

Genomic DNA was extracted from 65 mg of bacterial pellet. The extraction of each sample was performed by QIAamp DNA Mini kit (QIAGEN, Germany) according to the manufacturer's instructions. Further, to verify successful DNA extraction, electrophoresis was performed on 0.8% (w/v) agarose gels before it was used as a template for PCR amplification. PCR was conducted to amplify the full-length 16S rRNA gene fragment using universal bacterial primers 27FA (5′-AGA GTT TGA TCATGGCTAG-3′) or 27FC (5′-AGA GTT TGA TCCTGGCTAG-3′) and U1492R (5′-GTTACCTTGTTACGACTT-3′). The PCR protocol was initiated at 94°C for 2 min, followed by 30 cycles of denaturation at 94°C for 60 sec, annealing at 45°C for 60 sec and extension at 72°C for 60 sec, and a final extension at 72 °C for 15 min. The purification of the PCR product was performed by gel elution method using QIAquick Gel Extraction Kit (QIAGEN, Germany) as per manufacturer's protocol. The quantity and quality of the DNA extracts was checked by spectrophotometry and electrophoresis on 1% agarose gel. DNA was stored at - 20°C until use.

2.6 16S rRNA gene Sequencing

The partial nucleotide sequence was performed at 1st BASE (Malaysia) using Sanger method using 27FA or 27FC or U1492R primers. Identity of obtained sequence was determined by BLAST analysis at NCBI database. 16S rRNA sequences from the closest matched bacteria for each stain were aligned with Clustal W and phylogenetic trees were generated using the UPGMA ("Unweighted Pair Group Method with Arithmetic Mean).

2.7 Soil experiment

A soil kinetic experiment was done to evaluate the N-fixing and P-solubilizing efficiency of the isolated bacterial strains. Representative and composite soil samples were collected from a typical alluvial soil of Assam. Collected soil samples were air dried, sieved initially through 2 mm followed by an 80 mesh sieve, and then sterilized thrice in autoclave at 103421.36 N/m^2 for 15 min before inoculating bacterial strains. The bacterial strains were grown in Luria Bertaini media and incubated for 24 hrs at 28°C in a shaking incubator. 20 ml Broth of each strain was applied to sterilized soil samples weighing 200 g each. Azospirillum and Phosphobacteria were taken as positive control for N-fixing and P-solubilizing microbes respectively and were added as per manufacturer's protocol (Mani Dharma Biotech Pvt. Ltd, Chennai). Analysis of parameters like pH, easily mineralizable-N, Total Kjeldahl-N, urease (for N-fixing strains) Available-P and phosphatase (for P-solubilizing strains) were estimated periodically at 0, 7, 14, 21, 28, and 35 days respectively following Page et al. (1982). The experiment was conducted under ambient temperature 25±2°C and relative humidity 55% - 65%.

2.8 Crop trial:

Vermiconverted mixtures of organic wastes (USW) were applied to experimental soil (typic: endoaquepts) with a test crop of Rice (*Oryza sativa L*: variety Ranjit). The experiment was conducted in a randomized block design with three replicates during wet or Kharif season. Various combinations of vermicomposted organic waste were applied to all the plots keeping other management practices identical. Basic soil was collected and studied prior to application of treatments. The soil of the treated plots and the plant samples were measured at different growth stages of the crop. The treatment combinations used for the study were as follows:

T1 - NPK100 = 100% recommended NPK

T2- NPK100+FYM = 100% recommended NPK & Farmyard manure @ 10 t ha^{-1}

T3 - NPK100+VCei = 100% recommended NPK & *Eisenia* vermicomposted USW (4:1) @ 10 t ha^{-1}

T4 - NPK100+VCmp = 100% recommended NPK & *Metaphire* vermicomposted USW (4:1) @ 10 t ha^{-1}

T5 - NPK80+VCei = 80% recommended NPK & *Eisenia* vermicomposted USW (4:1) @ 10 t ha^{-1}

T6 - NPK80+VCmp = 80% recommended NPK & *Metaphire* vermicomposted USW (4:1) @ 10 t ha^{-1}

T7 - NPK60+VCei = 60% recommended NPK & *Eisenia* vermicomposted USW (4:1) @ 10 t ha^{-1}

T8 - NPK60+VCmp = 60% recommended NPK & *Metaphire* vermicomposted USW (4:1) @ 10 t ha^{-1}

T9 - NPK80+FYM = 80% recommended NPK & Farmyard manure @ 10 t ha^{-1}

T10 - NPK60+FYM = 60% recommended NPK & Farmyard manure @ 10 t ha^{-1}

T11 -VCei = *Eisenia* vermicomposted USW (4:1) @ 10 t ha^{-1}

T12 - VCmp = *Metaphire* vermicomposted USW (4:1) @ 10 t ha^{-1}

2.9 *Statistical analysis*

One-way ANOVA was performed to analyze real differences between various treatments by following standard method for randomized Block Design during the first year. Two-way ANOVA was performed for the crop trial by following standard method for randomized Block Design. Finally for identifying optimum treatment combinations, Least Significant Difference (LSD) test have been implemented.

3. RESULTS & DISCUSSION:

First year

3.1. Characterization of USW

Table 1 depicted that the USW sample was alkaline in nature with the presence of organic matter in the solid waste. The higher EC of the sample revealed the lower level of salinity in the USW, an essential character of good bio-compost better for crop growth. Besides important physical parameters like water holding capacity, porosity, cation exchange capacity was found to be significantly high. However, the status of availability of all the three major nutrients in USW viz., N, P and K were also found to be on higher side. It was evident from the table that USW contained considerably high total concentration of metals viz. Fe, Cu, Mn, Zn, Cr and Ni, which could be a serious concern with respect to environmental view points.

Table1. Physico-chemical characterization of Tezpur solid waste (USW)

Parameters	USW
pH	8.2±0.86
Conductivity(μS cm $^{-1}$)	37±1.89
Bulk density(g cc $^{-1}$)	0.754±0.08
Water Holding Capacity (%)	72.74±6.75
Porosity (%)	32.76±3.1
Particle density(g cc $^{-1}$)	1.09±0.14
Cation Exchange Capacity (meq 100g $^{-1}$)	30.69±2.7
TOC (%)	17.84±1.4
Total N (%)	4.57±0.56
Available P (mg kg $^{-1}$)	119.67±9.88
Available K (mg kg $^{-1}$)	117.5±10.6
*** Total and exchangeable metals in USW in ppm**	
Total Fe	5506.5±0.52
Exch. Fe	11.84±0.01
Total Cu	119.44±0.04
Exch. Cu	2.48±0.04
Total Mn	-------
Exch. Mn	5.6±0.26
Total Zn	284.6±0.02
Exch. Zn	14.8±0.26
Total Pb	131±0.5
Exch. Pb	0.36±0.002
Total Cr	20.14±0.02
Exch. Cr	-------
Total Ni	15.4±0.1
Exch. Ni	-------

3.2. Vermi-technology and lab-scale study:

The availability of exchangeable NPK in various treatments under vermicomposting with two different earthworm species viz. *Eisenia fetida* and *Metaphire posthuma* during the period of incubation is shown in Table 2. Interestingly, vermicomposting with both the species showed significant result in terms of N mineralization irrespective of treatment combinations. This may be due to increase microbiological activities in the vermicomposted treatments, which led to considerable increment in the amount of easily mineralizable nitrogen in this series. Earthworms actually enhance microbial activity (Fracchia *et al.,* 2006; Lazcano *et al.,* 2008). Some nitrogen is also added by the worms during vermicomposting in the form of mucus, nitrogenous excretory substances, hormones and enzymes (Hobson *et al.,* 2005; Suthar, 2006).

Similarly, P solubility increased over time in the same manner irrespective of treatment combinations under vermicomposting. Products of vermicomposting had a higher phosphorous content as compared to the products of traditional aerobic composting. This is corroborated by the findings of other workers who have observed stimulating effect of earthworms on availability of phosphorous in soil (Krishnamoorthy, 1990; Kaviraj and Sharma, 2003; Tognetti *et al.,* 2005). Satchell and Martin (1984) suggested that worm gut enzymes had a stimulating effect on phosphate solubilising bacteria. Bayon and Binet (2006) correlated the rise in phosphate content of vermicompost to presence of alkaline phosphatases in the worm casts.

The highest increase in K status has been found in vermicomposting of *Eisenia fetida* with USW only (469.06 mg/kg). Kaviraj and Sharma (2003) have reported that enhanced number of microflora in the gut of earthworm might have played an important role in increasing the potassium content during the vermicomposting process. Significant differences among the treatment combinations were observed under both the earthworm species adopted for waste transformation throughout the study.

Table2. Nutrient availability in vermicomposted and composted USW

Eisenia fetida			
	N(mg/kg)	**P(mg/kg)**	**K(mg/kg)**
USW	324.8±28	125.13±3.15	469.06±18.09
USW+CD,2:1	1131.2±5.6	192.86±8.48	435.46±53.07
USW+CD,4:1	1148±14	190.83±15.30	440.26±8.92
CD	938±28	638.42±27.31	403.93±8.20
Metaphire postheuma			
	N(mg/kg)	**P(mg/kg)**	**K(mg/kg)**
USW	1064±28	162.94±31.94	403.6±24.75
USW+CD,2:1	1106±42	190.23±33.73	464.66±22.78
USW+CD,4:1	1260±70	226.41±26.27	456.26±22.98
CD	1274±24.24	265.03±19.29	407.46±10.32
Composting			
	N(mg/kg)	**P(mg/kg)**	**K(mg/kg)**
USW	851.2±5.6	122.03±13.25	495.53±52.75
USW+CD,2:1	980±14	173.45±21.71	489.06±36.70
USW+CD,4:1	1134±14	175.34±14.14	486.13±2.19
CD	910±14	310.78±34.23	505.6±36.24

The carbon content in the vermicomposted samples are utilized as the source of energy for earthworms. The value of C for all treatment combinations range from (13.6% - 27.84 %). Generally the total organic carbon in all the treatments showed a declining trend as the decomposition progressed (fig 1). The combined action of earthworms and microorganisms may be responsible for reduction of carbon from the vermicompost in the form of CO_2 in respiration and mineralization of organic matter. Moreover, excreta and body fluid of earthworms encourage microbial multiplication which in turn promotes rapid respiration that minimizes the carbon level of the waste (Suthar, 2007).

Fig1.Changes in total organic carbon (TOC) during vermicomposting with *Eisenia fetida*, *Metaphire postheuma and Composting*

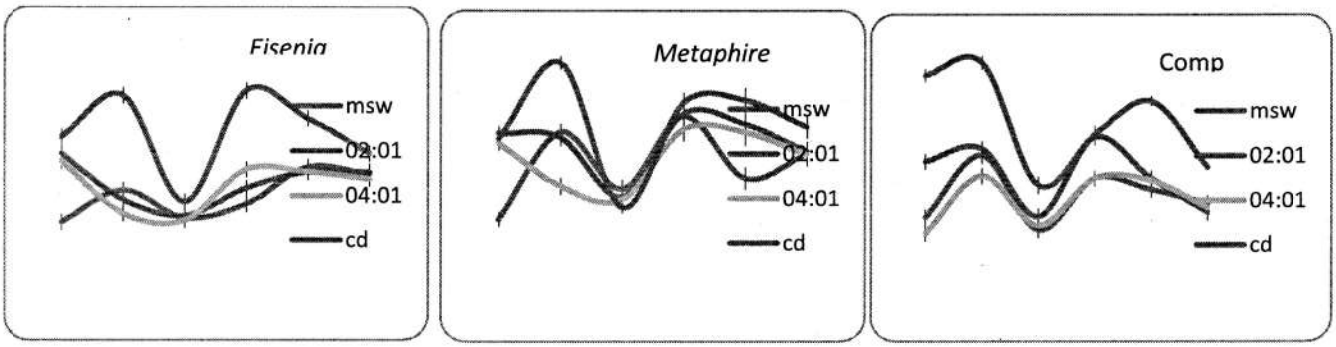


Table 3: Impact of vermicomposting on bacterial growth in USW mixtures (mean± standard deviation).

Treatment	No. of bacteria/ml ($x10^5$)
*VC: USW + CD (4:1) (*Metaphire postheuma)*	**82±4.21**
VC : USW + CD (4:1) (*Eisenia fetida)*	**75±5.8**
Compost : USW + CD (2:1)	**67±3.7**
P value (0.05)	**0.002**

*** VC: Vermicompost**

The result on total bacterial count in composted and vermicomposted USW mixtures (table -3) demonstrates highly significant differences (p = 0.002). Bacterial population was higher in vermiconverted samples as compared to composted samples. Increase in microorganisms may have largely contributed towards accelerated nutrient availability under vermicomposting. Interestingly, USW + CD (4:1) found to be a good substrate for microbial growth, strongly substantiating the findings of Suthar (2009) and confirming the advantage of vermicomposting.

Second year

3.3. Characterization of vegetable waste, rice straw and cowdung:

The physico-chemical properties of the three organic residues are presented in Table 4. All these materials were neutral in reaction with high total organic carbon (TOC) and Total Kjeldahl nitrogen (TKN) levels but with low soluble Nitrogen. Other Carbon fractions like Fulvic Acid carbon (FAC) and Humic acid carbon (HAC), which are considered as humification indicators, were substantially high in all the three organic waste components. On the contrary, bulk density (BD) of these residues was low indicating their porous texture. However, MBC was higher in RS and VW as compared to CD. But both bioavailability of available phosphorus and exchangeable potassium was conspicuously higher in VW and CD compared to RS.

Table 4: Basic properties of rice straw, vegetable waste and cow dung used for the study.

Parameters	Rice Straw	Veg. waste	Cowdung
Available Nitrogen (mg/kg)	77.33±70.46	96±56	40±56
Total nitrogen (%)	17.017±0.24	15.92±0.12	15.4±0.24
Available K (mg/kg)	304.6±101.53	657.6±219.2	812±270.66
Available P (mg/kg)	138.07±0.40	272.12±0.40	274.53±0.40
Microbial respiration (µg CO2/g/hr.)	4.074±0.51	3.94±0.98	1.99±0.49
Bulk density (g/cc)	0.46±0.000	0.57±0.000	0.49±0.000
pH	7.1±0.1	7.5±0.1	7.6±0.1
TOC (%)	6.75± 0.06	5.14±0.09	6±0.12
FAC (%)	2.33±0.09	1±0.12	2.5±0.06
HAC (%)	3.83±0.09	2.52±0.09	4.08± 0.09

3.4. Changes in pH, easily mineralizable N, available P and exchangeable K:

Table 5 depicts the changes in pH and availability of N, P and K in various treatments during the period of incubation. Interestingly, vermicomposting with both the species showed significant result in terms of N mineralization irrespective of treatment combinations. This may be due to increase microbiological activities in the vermicomposted treatments, which led to considerable increment in the amount of easily mineralizable nitrogen in this series. Some nitrogen is also added by the worms during vermicomposting in the form of mucus, nitrogenous excretory substances, hormones and enzymes (Hobson *et al.,* 2005; Suthar, 2006).Satchell and Martin (1984) suggested that worm gut enzymes had a stimulating effect on phosphate solubilising bacteria. Interestingly, P content decreased initially (30 d) but increased substantially at the end of bio composting process. Highest P content recorded inT4 followed by T10 and T9. This may be due to the earthworm gut phosphatise enzyme that accelerates P solubility and the P-solubilizing microorganisms contributed through the vermicasts (Suthar et al.,2012; Sahariah et al.,2014).

The K content showed highest activity in 30 days.. Significantly high K availability was recorded under T2. Earthworm process materials with high concentration of K due to enhanced microbial and enzyme activities in the gut of earthworms (Tripathi and Bhardwaj, 2004).

Table 5: Periodic changes in pH, Available N, P and K during biocomposting

Treatments	pH		AN		AP		EK	
	Days		Days		Days		Days	
	0	60	0	60	0	60	0	60
E(CD)	7.3	7.4	100.8	560	41.1	189.5	790.2	712.9
C(CD)	7.7	6.8	126.9	280	45.6	215	852.4	866.8
P(CD)	7.7	7.3	115.7	93.3	35.6	186	626.8	698.5
E(CD+VW)	7.7	7.3	137.2	84	44.4	227.5	1276.2	656.3
C(CD+VW)	7.6	7.1	112	476	43.9	212.9	457.8	416.9
P(CD+VW)	7.4	7.3	114.8	616	45.9	189.3	620.6	587.8
E(CD+RS)	7.2	6.3	119.6	594.7	42.6	89.7	567.1	641.7
C(CD+RS)	7.0	6.4	115.7	364	56.7	88.1	214.4	390.9
P(CD+RS)	7.2	6.5	114.8	289.3	55.9	201.2	230.4	462.0
E(CD+VW+RS)	7.3	6.4	126	377.3	55.7	198.4	559.7	522.7
C(CD+VW+RS	7.4	6.1	112	224	53.7	117.1	375.8	422
P(CD+VW+RS)	7.3	6.1	129.7	252	53.2	114.7	360.7	422.1
LSD	0.25		34.5		7.81		O.11	
P value	<0.005		<0.005		<0.005		<0.005	

3.5 Dynamics of carbon fractions during vermicomposting:

Table 6 depicts the dynamics of TOC, FAC, HAC in various treatments during the incubation. Gradual increase in FAC and HAC was observed during vermicomposting process with significant reduction in TOC. This may be due to slowing down of microbial activity in the later phase. Our findings are in good agreement with Deka et al., (2011). Fulvic acid C on the contrary increased initially and decreased later (30 to 60 days), but, humic acid C radically increased between 30 to 60 days of incubation. High HAC coupled with low TOC indicates richness of humic substances in the end product and also specify the maturity of the composting process at 60 days. Therefore, this trend justified that mineralization rate culminated between 30 to 60 days of incubation. Moreover, high FAC and HAC are significant indicators of high quality compost (Campitelli and Ceppi, 2008).

Table 6: Periodic changes in TOC, FAC and HAC during vermicomposting

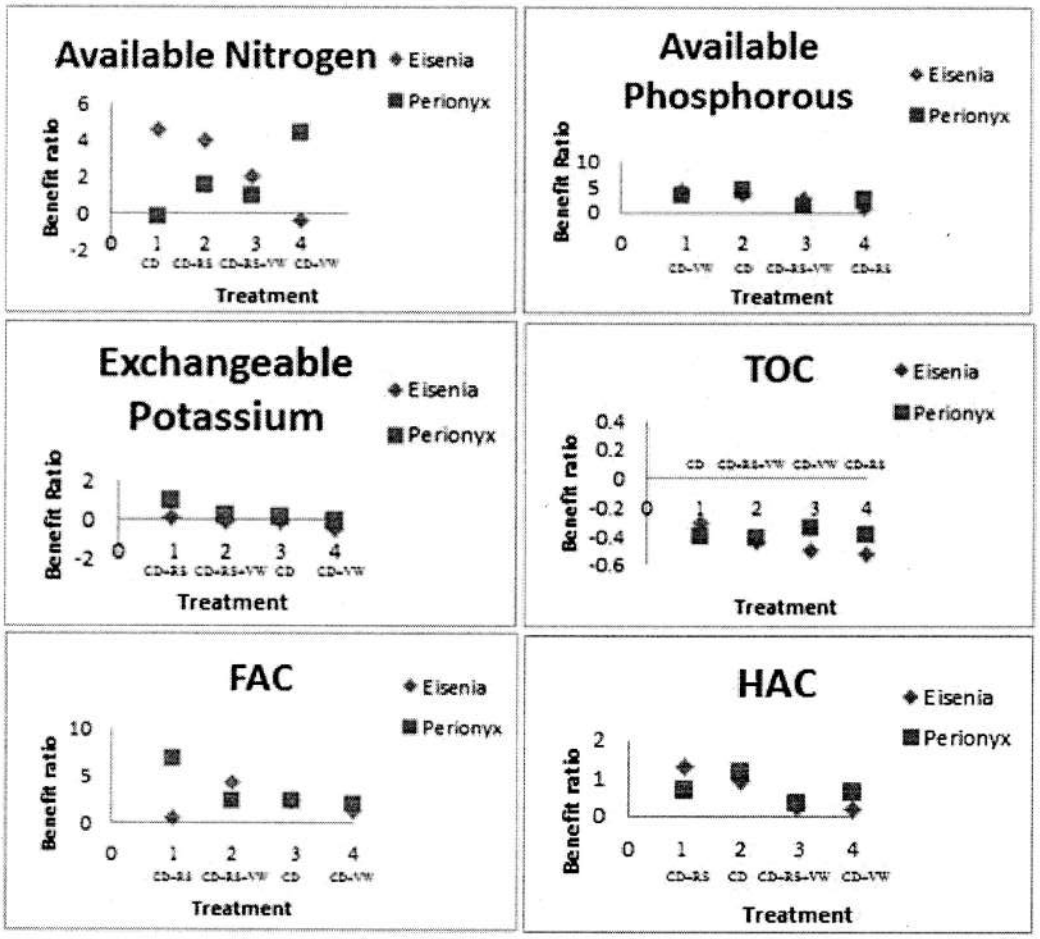
Treatments	TOC		FAC		HAC		DH	
	Days		Days		Days		Days	
	0	60	0	60	0	60	0	60
E(CD)	7.5	5.14	1.58	5.4	3.4	6.6	1.05	1
C(CD)	8.3	6.8	2.5	5.3	4.1	6.3	1	1
P(CD)	8.3	4.9	1.58	5.3	2.8	7	1.17	1
E(CD+VW)	8.3	4.1	1.95	4.4	4.7	5.1	1	1
C(CD+VW)	7.9	7.9	1	5.4	2.5	6	1	1
P(CD+VW)	7.8	5.1	1.43	4.3	4.9	5.5	1	1
E(CD+RS)	10.1	4.8	2.52	4.2	3.4	7.8	1	1
C(CD+RS)	9.5	7.1	2.33	5	3.8	5.3	1	1.04
P(CD+RS)	9	5.4	0.62	4.9	4.1	6.9	1	1
E(CD+VW+RS)	9.4	5.2	1	5.2	5.4	6.3	1	1.04
C(CD+VW+RS	10.1	5.7	1.87	4.2	5	5.5	1	1
P(CD+VW+RS)	10.1	5.8	1.25	4.2	4	6.5	1	1
LSD	0.74		0.08		0.76			
P Value	<0.005		<0.005		<0.005		<0.005	

3.6 Comparison of *Eisenia fetida* and *Perionyx excavates* in terms of nutrient enhancement and compost maturity:

Fig 2 presents a comparative analysis of the benefit of vermicomposting with two different earthworm species with respect to nutrient availability and compost maturity. *Eisenia* vermicompost systems performed more efficiently than *Perionyx* system with respect to N availability in CD, CD+RS and CD+RS+VW mixtures. In contrast, *Perionyx* vermicompost system was noticeably beneficial for CD+VW mixture. Similar results were also obtained with respect to P availability in CD+VW and CD+RS+VW mixtures. On the other hand, benefit in K availability was negligible in both the systems for CD, CD+RS+VW and CD+RS mixtures. However, positive benefit ratio was only obtained under *Perionyx* system in CD+RS mixture.

As reduction in TOC level reflects the proficiency of the composting process, hence, we considered the extent of reduction in 60 days to compute the benefit ratio for TOC. Interestingly, both the systems showed similar TOC reduction ability in CD and CD+RS+VW mixtures, whereas, *Eisenia* system was slightly superior over *Perionyx* system in CD+VW and CD+RS mixtures. However, such results were varied for the humified fractions of organic C (i.e. FAC and HAC). Highest benefit ratio for FAC and HAC was achieved for *Eisenia* system in CD+RS mixture. On the other hand, *Perionyx* system was noticeably superior in CD+VW and CD+RS mixtures.

Fig 2 Relative beneficial impact of two vermicomposting systems with respect to nutrient availability and compost maturity:



3.7 Isolation of bacterial colonies:

Number of colonies observed under the treatments of 60 days interval at 10^{-6} dilution were recorded (Table 7). A total of nine bacterial colonies were isolated from the treatments (Fig3). Colonies observed in Pikovskaya Agar medium were T1A2P, T1A3P, T1A6 B , T6A3P, T10A6P, T11A6P, T12A6P. The morphology of T1A2P, T1A3P, T1A6P isolated from Cowdung(*Eisenia*) were circular and rose. T6A3P and T11A6P isolated from Vegetable waste + Cowdung (*Perionyx*) and Vegetable + Ricestraw + Cowdung(compost) respectively were raised and circular shaped. T10A6P and T12A6P isolated from Vegetable + Ricestraw + Cowdung (*Eisenia*) and Vegetable + Ricestraw + Cowdung (*Perionyx*) respectively were circular and flat shaped. Morphology of colonies T6A6B and T7A6B

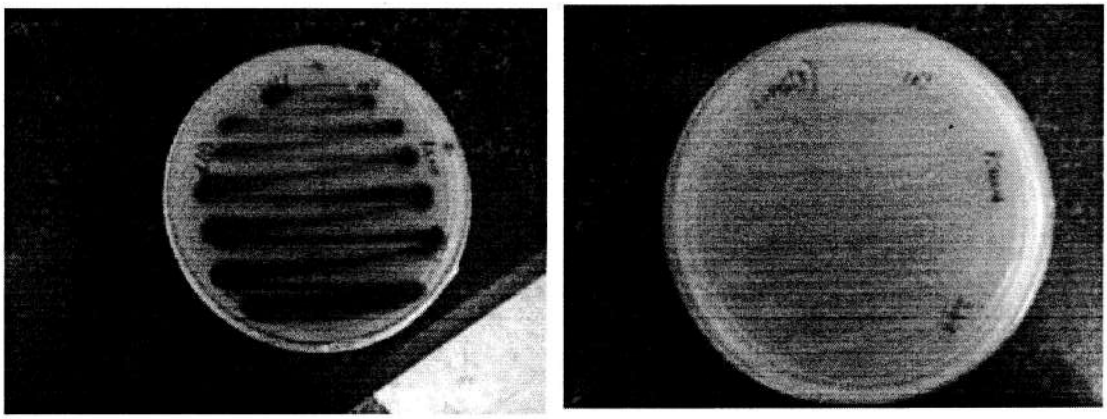
isolated fromVegetable waste + Cowdung(*Perionyx*) and Ricestraw + Cowdung(*Eisenia*) respectively were observed to be punctiform and flat.

Table 7: Bacterial count in the biocomposted samples

Treatments	No. of colonies per ml	
	Burk's medium	Pikovskaya agar medium
T1 CD(E)	3.52×10^3	3.86×10^3
T2 CD(C)	5.92×10^3	6.96×10^3
T3 CD(P)	3.52×10^3	3.86×10^3
T4 CD+VW(E)	1.92×10^3	1.70×10^3
T5 VW+CD(C)	5.04×10^3	5.56×10^3
T6 VW+CD(P)	2.64×10^3	2.64×10^3
T7 RS+CD(E)	2.80×10^3	2.96×10^3
T8 RS+CD(C)	2.62×10^3	1.64×10^3
T9 RS+CD(P)	9.9×10^2	1.01×10^3
T10 VW+RS+CD(E)	4.73×10^3	1.95×10^3
T11 VW+RS+CD(C)	1.28×10^3	9.1×10^2
T12 VW+RS+CD(P)	1.01×10^3	1.06×10^3

Figure 3: Pictures of plates containing isolated bacterial strains

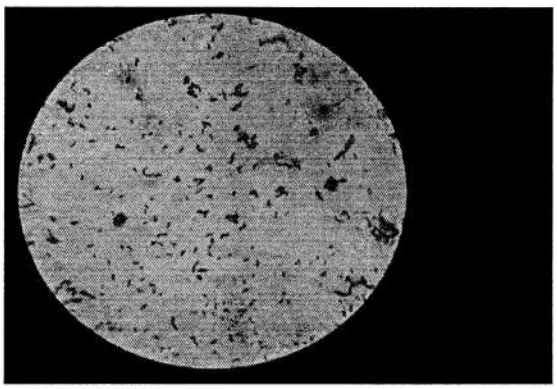
T1A3P **T10B1P**



3.8 Characterization of the bacterial strains:

The bacterial strains were characterized according to Bergeys manual of determinative bacteriology. The morphological characterization were done of the following treatments and the results are as follows:T1A3P - Gram positive, rod; T1A2P - Gram negative, rod ; T10A6P - Gram negative, rod ; T11A6P - Gram negative, rod.

Figure 4: Strains showing Gram Positive result



T1A3P

3.9 Biochemical characterization

Table 8 shows the biochemical characterizations of different strains isolated from potential feed mixtures which showed high P-solubilizing and N-fixing ability. In general, the CD only, CD+RS, and CD+RS+VW feed stocks were found to be most compatible for the selected earthworm species. Among the isolated strains positive results for starch hydrolysis test were shown by T6A3P and T7A6B indicating that the strain used starch as a source of carbon accomplished by the enzyme α-amylase. For Methyl Red test positive results were observed in T1A6P, T6A3P, T6A6B and T7A6B. It determined that these strains could oxidize glucose with production and stabilization of high content acid product. On the contrary, all the results were found to be negative for Casein Hydrolysis test indicating that these strains could not produce casease to hydrolyze casein. Hence, the study confirmed that the bacterial colonies isolated from T1A3P, T1A6P, T7A6B, and T10A6P had diversified potential apart from N fixing or P solubilizing ability (Table 8).

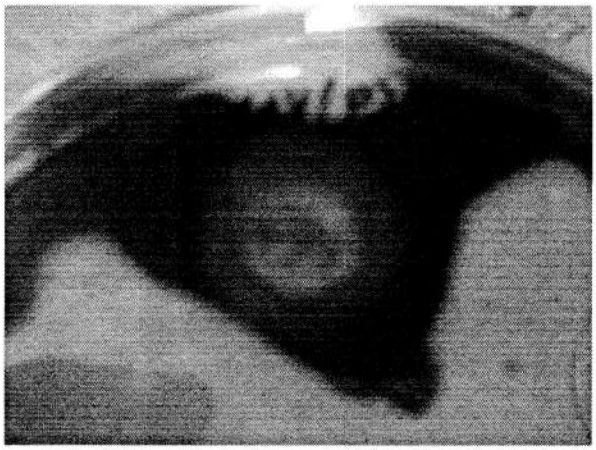
Table 8: Biochemical profiling of the vermicompost samples:

The bacterial isolates that showed prolific N-fixing and/or P-solubilizing efficiency during vermicomposting were selected from different biochemical analysis to explore their greater potential.

Treatment	Dilution	Starch hydrolysis test	Methyl red test	Casein hydrolysis test
T1A3P	10^{-3}	Negative	Negative	Negative
T1A6P	10^{-6}	Negative	Positive	Negative
T6A3P	10^{-3}	Positive	Positive	Negative
T6A6B	10^{-6}	Negative	Positive	Negative
T7A6B	10^{-6}	Positive	Positive	Negative
T10A6P	10^{-6}	Negative	Negative	Negative
T11A6P	10^{-6}	Negative	Negative	Negative
T12A6P	10^{-6}	Negative	Negative	Negative

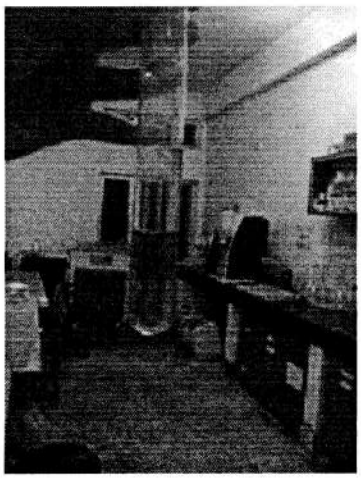
Figure 5: Results of biochemical characterization:

Starch Hydrolysis test



T6A3P

Methyl Red Test



T6A6P

Third year

3.10 Earthworm study

Fig 6 shows the changes in *Eisenia* body length in different combinations of three different earthworms over various durations (0, 30, 60 days).While E+P and E+P+M combinations showed similar trend of changes in body length E+M combination showed a different one. In case of E+M unlike two other combinations changes in body length was found higher in 30 days than two other days of observation.

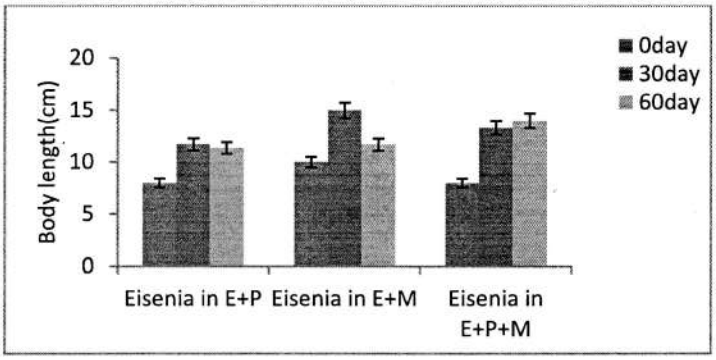


Fig 6: Body length of *Eisenia* in three treatments

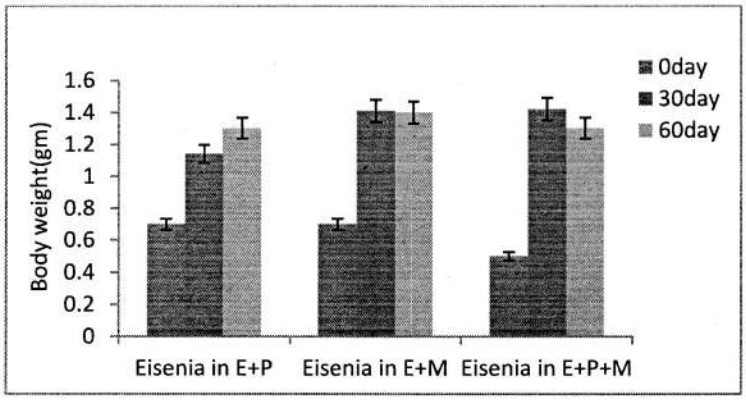


Fig 7: Body weight of *Eisenia* in three treatments

Fig 7 shows the changes in *Eisenia* body weight in different combinations of three different earthworms over various durations (0, 30, 60 days). While E+P and E+P+M combinations showed similar trend of changes in body weight E+M combination showed a different one. In case of E+M unlike two other combinations changes in body weight was found higher in 30 days than two other days of observations.

Fig 8 shows changes in body length of *Metaphire* in three combinations of various duration of (0,30,60 days). There is a similar trend in all the three combinations. Body length of *Metaphire* is found to be higher in 30days.

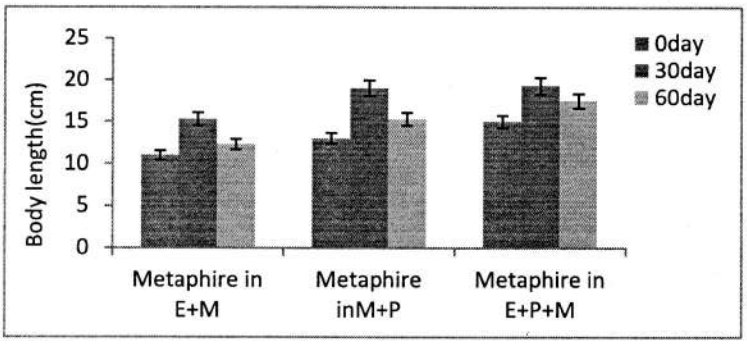


Fig 8: Body length of *Metaphire* in three treatments

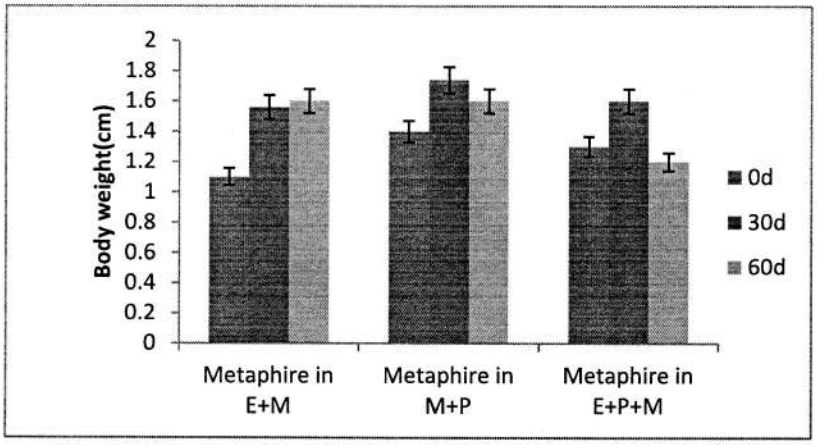


Fig9: Body weight of *Metaphire* in vermicomposting

Fig 9 shows the changes in *Metaphire* body weight in different combination of three earthworm over various duration (0,30, and 60 days). There is no clear cut trend in any of the treatments. In general the 30 days values are higher in comparison to 0 and 60 day.

Fig10: Body length of *Perionyx* in three vermicomposting

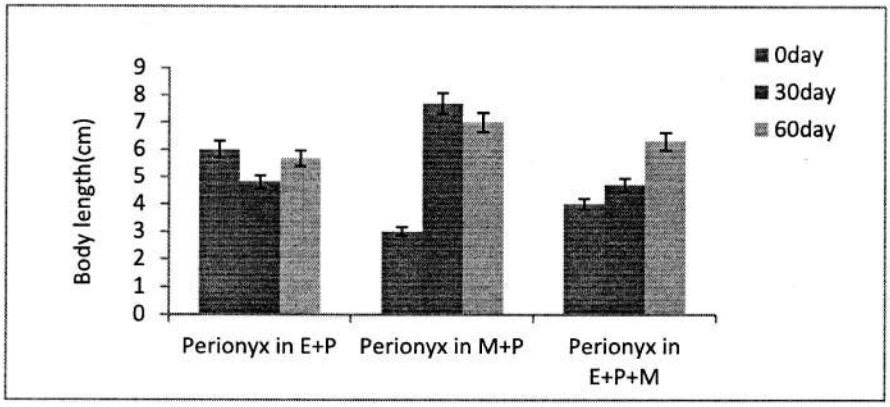


Fig 10 shows the changes in *Perionyx excavates* earthworms body length in different combinations of three different earthworms over various durations (0, 30, 60 days).There was a sharp increase of P*erionyx* length in (M+P) combination during 30 days observation.

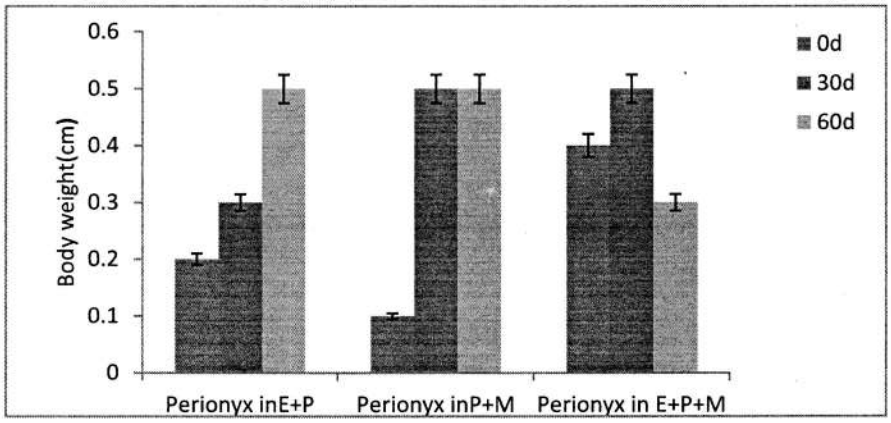


Fig 11: Body weight of *Perionyx*

Fig 11 shows the changes in *Perionyx* earthworms body weight in different combinations of three different earthworms over various durations (0, 30, 60 days).There is an increase in body length of *Perionyx* in both the combinations of E+P and P+M but in the combination of E+P+M, body length of 30days increased gradually while it decreased in 60days .

3.11 Changes in bioavailability of TKN, Available N & P during biocomposting:

Fig 12 and Fig 13 represents the results on changes in TKN and Available N. TKN content increased during biocomposting. There is an increase content in nitrogen from 0 to 60 day in all the treatments. Enhancement of total nitrogen content is higher in the treatment of three earthworm mixtures (*Eisenia fetida*, *Perionyx excavates*, *Metaphire posthuma*). Many factors such as N status of the feed mixture; excretory products, mucus, body fluid, enzyme and even decaying tissue of the death earthworms are associated with the higher level of N in Vermicomposting (Suthar,2009b; Deka et al.,2011).

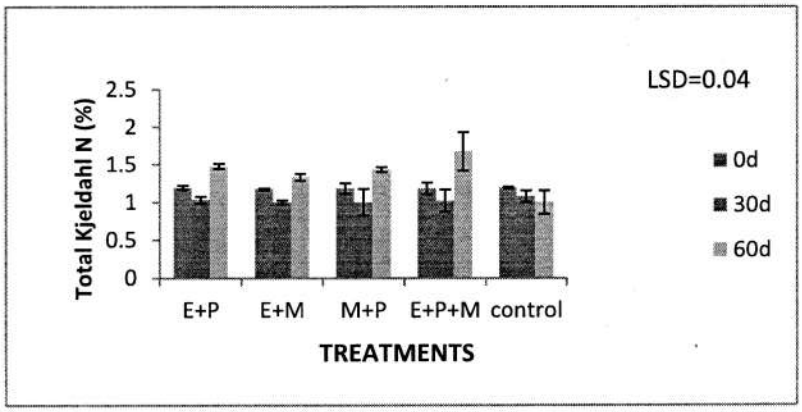


Fig 12:Changes in TKN during the period of incubation

However in response to nitrogen availability, both *Eisenia fetida* and *Perionyx excavates* treated combination responded well. In three earthworm combinations N availability increased sharply (LSD=45.8). N availability was high compared to the controlled conditions. Earthworm enhances N content through addition of excreta and secretion of polysaccharides and promotes microbial activity in the substrate by modifying the substrate microclimate (Sahariah et al., 2015).

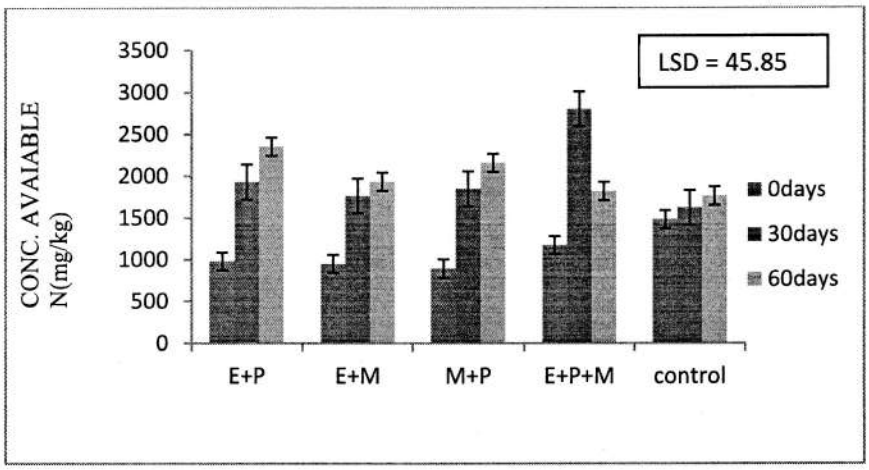


Fig 13: Periodic changes mineralizable N (mg/kg) during the period of incubation.

Phosphorus content decreased initially but increase substantially at the end .(Fig14). The influence of earthworm activity on phosphorus was more pronounced, there is an increased trend P content in first combination followed by third and second (LSD=2.36) .The availability of P in the end product may vary depending upon the earthworm metabolism and it was suggested that the release of phosphorus in available form is contributed partly by earthworm gut phosphatase,

and further release of P through P-solubilizing microorganisms present in worm casts (Suthar, 2012). Studies indicate the highest plant available forms of P in vermicomposted wastes mainly due to activities of P-solubilizing bacteria and enzymatic activities of earthworm gut (Suthar et al, 2012).

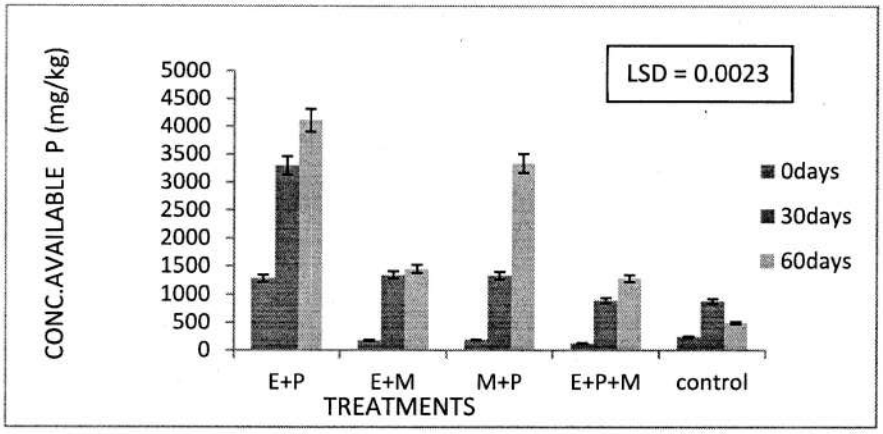


Fig 14: Available P (mg/kg) concentration at 0 day, 30 days and 60 days

3.12 Molecular characterization of bacterial isolates:

Differentiative staining of bacterial strains isolated from earthworm gut and earthworm consortium feed mixtures was performed. Among the eight stains isolated from the earthworm gut two gram positive (Strain # 12 and 14) and six gram negative bacteria (Strain # 2, 8, 9, 10, 11, and 13) were observed. The three strains isolated from earthworm consortium feed mixtures were all found to be gram negative bacteria (Strain # 3, 4, and 5) and the one isolated from the soil is found to be gram positive (Strain # 1).

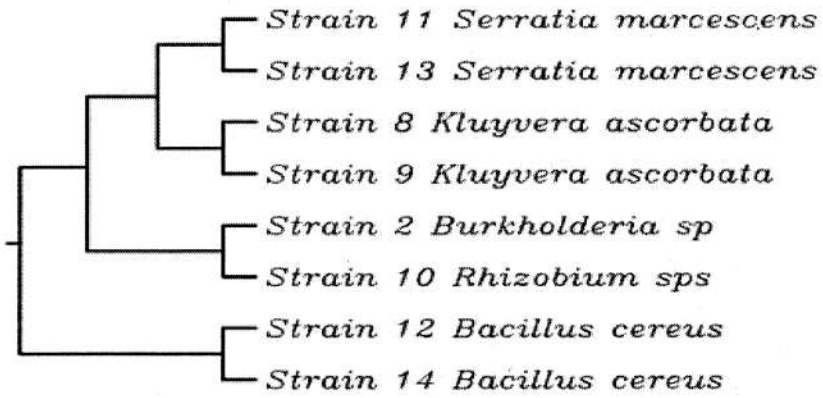
Table 9: The details of molecular information of the isolated bacterial strains

Strain No.	Differential stain	**Sequence length used (base) for analysis**	**Accession No. of deposited sequence**	**Name of closest matched organism**	**Percentage identity with the closed match**
1	Gram Positive	808	KU321341	*Bacillus vietnamensis* strain M4J10	100%
2	Gram Negative	623	KU321342	Burkholderia sp. Gan-DAB-FL5	99%
3	Gram Negative	913	KU321343	*Kluyvera ascorbata*	99%
4	Gram Negative	317	KU321344	*Burkholderia contaminans* strain RA_14B.2	100%
5	Gram Negative	981	KU321345	*Kluyvera ascorbata* partial	99%
8	Gram Negative	956	KU321346	*Kluyvera ascorbate* partial	99%
9	Gram Negative	970	KU321347	*Kluyvera ascorbate* partial	99%
10	Gram Negative	989	KU321348	Rhizobium sp.	99%
11	Gram Negative	1002	KU321349	*Serratia marcescens*	99%
12	Gram Positive	1024	KU321350	Bacillus cereus strain RNS_01	99%
13	Gram Negative	1003	KU321351	*Serratia marcescens* strain S823	99%
14	Gram Positive	1003	KU321352	*Bacillus cereus* strain MYSP90	100%

Complete 16S rRNA gene was amplified from all the twelve isolates and were partially sequenced. Obtained sequence was used for molecular identification of the isolates. Table 9 summarizes the obtained sequencing results and identity of the isolates. Sequences used for analysis were deposited in NCBI 16S rRNA database and obtained accession numbers are listed in the table. Phylogenetic analysis (Fig 15) of the bacterial isolates from earthworm gut represents close relation

of strain # 11, 13, 8 and 9. Strain # 2, and 10 appears distant from the first group. Whereas stain # 12 and 14 forms a group which is distant from previous two groups.

Figure 15: Phylogenetic tree of the isolated bacterial species from earthworm gut



3.13 Effect of isolated NFB and PSB strains on N and P availability in soil:

To assess the bio-fertilizer potential of the isolated bacterial strains, the formulations of the pure cultures of the respective strains were applied to sterilized soil. In the PSB inoculated soil samples, pH shifted from acidic to neutral range over time irrespective of strains. However, the extent of such pH shift was significantly prominent in strain14 followed by strain2 as compared to the other strains and Phosphobacteria(Table 11). As observed for the PSB strains, the soil inoculations with NFB strains also significantly shifted the soil pH towards neutrality as compared to Azospirillum(Table13). Interestingly, total organic carbon content of the soil samples increased initially under all the PSB inoculated soil as compared to the control which reduced substantially at the 35th day. Similar trend was also observed in NFB inoculated soil samples. At the end of the experiment, TOC content was significantly high in NFB strain 8 followed by NFB strain 1,strain 2 and strain 9 as compared to others whereas in PSB inoculated soils highest TOC content was recorded in strain 11 inoculated soil followed by strain 2 inoculated soil. However, the initial trend of TOC accumulation signifies rapid bacterial proliferation which in turn slowed down the carbon mineralization process till 28th day. Interestingly, the reduction in TOC indicates the acceleration of carbon mineralization process due to

microbial action in the soil (Janzen, 2004) which promotes bioavailability of essential nutrients like nitrogen and phosphorus.

Phosphorus availability significantly increased in soil treated with isolated PSB strains as compared to the control and Phosphobacteria inoculated soil(Table 12). Maximum increment of P availability was recorded under strain 2 followed by strain 14 and strain 5. Similarly phosphatase activity also significantly enhanced in strain 2 and strain 14 inoculated soil. This advocates that the PSB strains isolated from the earthworm intestine considerably enhanced P bioavailbility through activation of phosphatase. The results are in good agreement with our previous findings (Bhattacharya et al., 2013).

We isolated eight prolific N-fixing bacterial strains from the earthworm gut and vermicompost. In general, availability of mineralizable nitrogen significantly enhanced with soil treated with our isolates as compared to Azospirillum. The N availability in soil at the 28^{th} day of the experiment under various bacterial inoculates was in the order : strain 8 > strain10 > strain 9 > strain 12 > strain 13 > strain1 > strain 3 > Azospirillum > Control. However on 35^{th} day the available N content of the soil generally reduced under all treatments and was in the order strain 8 = strain9 strain10 = strain 13 > strain3 > strain 1 > strain 4 > Azospirillum > Control. On the other hand, total nitrogen content under various NFB inoculates considerably fluctuated over time during the whole period of the study(Table 14). Such fluctuations was in TKN was may be due to the influence of the bacterial inoculates on the N immobilization-mineralization dynamics in soil.Finally, the total nitrogen content in soil under various bacterial inoculates was in the order: Azospirillum = strain1 = strain 4 > strain 12 > strain 3 > strain10= strain13 > strain8 > strain9. This is interesting because the result shows that although the gradual buildup of nitrogen stock in soil was higher due to Azospirillum inoculation but the mineralization efficiency for the isolated bacterial strain was significantly higher than the Azospirillum. Thus both the PSB and NFB isolates have higher biofertilizer potential as compared to the readily available strains of Phosphobacteria and Azospirillum in the market.

Table 10.Changes in pH and TOC in P-solubilising microbes:

Parameters	Time	Control	Strain 2	Strain 5	Strain 11	Strain 14	Phosphobacteria
pH	0 D	5.60±0.00	5.63±0.00	5.60±0.00	5.63±0.06	5.63±0.06	5.60±0.00
	7 D	5.59±0.01	6.82±0.01	6.58±0.00	6.54±0.00	6.98±0.00	6.00±0.00
	14 D	5.58±0.00	6.82±0.00	6.88±0.00	6.74±0.17	7.12±0.01	6.03±0.05
	21 D	5.61±0.00	7.13±0.00	6.90±0.00	6.90±0.00	7.34±0.00	6.31±0.00
	28 D	5.62±0.01	7.28±0.01	7.03±0.00	6.94±0.01	7.44±0.04	6.44±0.04
	35 D	5.64±0.04	7.42±0.03	7.13±0.00	7.04±0.01	7.55±0.03	6.56±0.02
TOC(%)	0 D	0.71±0.03	0.71±0.06	0.75±0.03	0.71±0.03	0.71±0.06	0.71±0.03
	7 D	0.70±0.01	2.22±0.01	1.06±0.01	1.60±0.01	1.27±0.00	1.26±0.01
	14 D	0.75±0.00	1.44±0.02	1.37±0.02	1.48±0.02	1.31±0.02	1.28±0.04
	21 D	0.63±0.00	1.18±0.01	0.85±0.03	1.25±0.01	0.98±0.01	0.95±0.01
	28 D	0.60±0.01	1.31±0.03	1.21±0.03	1.38±0.01	1.17±0.03	1.23±0.01
	35 D	0.62±0.05	0.70±0.03	0.6±0.03	0.78±0.02	0.55±0.04	0.62±0.02
P(T)	0.00						
LSD(T)	0.01						

Table 11. Changes in Av P and Phosphatase in P-solubilising microbes

Parameters	Time	Control	Strain 2	Strain 5	Strain 11	Strain 14	Phosphobacteria
Av P(mg/kg)	0 D	69.20±0.02	69.21±0.04	69.20±0.02	69.21±0.04	69.20±0.02	69.23±0.02
	7 D	69.21±0.00	101.90±0.09	82.87±0.02	79.27±0.38	100.75±0.05	82.21±0.20
	14 D	38.80±0.07	262.36±0.02	82.64±0.09	73.36±0.07	115.94±0.09	77.60±0.20
	21 D	39.87±0.16	129.32±0.07	84.10±0.04	69.38±0.26	96.62±0.05	58.84±0.04
	28 D	47.71±0.54	223.76±0.11	125.63±0.14	84.45±0.27	178.94±0.12	82.21±0.09
	35 D	48.06±0.36	43.84±0.09	25.05±0.10	17.26±0.25	48.12±0.09	20.24±0.23
Phosphatase(µg/g)	0 D	1.31±0.00	1.22±0.15	1.4±0.15	1.35±0.06	1.31±0.26	1.22±0.15
	7 D	3.29±0.12	132.47±0.05	106.63±0.09	99.07±0.12	127.96±0.26	68.14±0.09
	14 D	11.05±0.00	208.76±0.02	106.99±0.09	98.51±0.28	131.32±0.23	71.36±0.04
	21 D	19.46±0.22	94.36±0.09	81.69±0.08	55.37±0.10	90.92±0.09	38.21±0.19
	28 D	26.45±0.12	234.39±0.16	167.41±0.08	140.79±0.03	228.55±0.06	102.55±0.06
	35 D	27.16±0.20	259.06±0.41	189.99±0.15	139.7±0.25	249.06±0.17	155.35±0.14
P value	0.00						
LSD(T)	<0.07						

Table 12. Changes in pH and TOC in N-fixing microbes:

Parameters	Time	Control	Strain 1	Strain 3	Strain 4	Strain 8	Strain 9	Strain 10	Strain 12	Strain 13	Azospirullum
pH	0 D	5.60±0.00	5.60±0.00	5.63±0.00	5.60±0.00	5.63±0.05	5.60±0.00	5.63±0.05	5.63±0.05	5.63±0.05	5.60±0.00
	7 D	5.59±0.01	6.70±0.00	6.67±0.00	6.70±0.00	6.68±0.00	6.93±0.00	6.53±0.00	6.91±0.00	6.84±0.00	5.60±0.00
	14 D	5.58±0.00	6.63±0.25	6.91±0.00	6.82±0.02	6.84±0.03	6.97±0.01	6.86±0.01	6.95±0.05	6.87±0.02	5.60±0.01
	21 D	5.61±0.00	7.14±0.04	7.14±0.03	7.07±0.01	7.11±0.02	7.21±0.11	7.06±0.01	7.12±0.02	7.04±0.04	6.57±0.05
	28 D	5.62±0.01	7.35±0.00	7.35±0.04	7.38±0.00	7.44±0.04	7.54±0.01	7.16±0.00	7.44±0.04	7.34±0.04	6.47±0.04
	35 D	5.64±0.04	7.40±0.01	7.38±0.01	7.36±0.01	7.46±0.02	7.54±0.04	7.22±0.01	7.52±0.02	7.43±0.02	6.67±0.04
TOC(%)	0 D	0.71±0.03	0.71±0.06	0.75±0.03	0.71±0.03	0.71±0.06	0.71±0.03	0.71±0.03	0.71±0.06	0.71±0.06	0.75±0.03
	7 D	0.70±0.01	1.61±0.01	1.51±0.01	1.29±0.01	1.55±0.03	1.31±0.03	1.29±0.03	1.52±0.03	1.50±0.00	1.65±0.01
	14 D	0.75±0.00	1.47±0.00	1.64±0.04	1.37±0.02	1.43±0.00	1.31±0.02	1.70±0.04	1.67±0.02	1.01±0.02	1.36±0.00
	21 D	0.63±0.00	1.05±0.01	1.20±0.00	1.13±0.00	1.29±0.00	1.21±0.04	1.09±0.01	1.12±0.01	0.91±0.03	1.05±0.01
	28 D	0.60±0.01	1.21±0.03	1.31±0.03	1.21±0.03	1.41±0.01	1.35±0.01	1.25±0.03	1.22±0.03	1.14±0.01	1.22±0.01
	35 D	0.62±0.05	0.97±0.02	0.97±0.04	0.92±0.04	1.03±0.05	0.97±0.02	0.89±0.05	0.89±0.07	0.90±0.05	0.87±0.07
P(T)	0.00										
LSD(T)	0.01										

Table 13. Changes in Easily minerizable N, Total kjeldahl N and urease in N-fixing microbes:

Parameters	Time	Control	Strain 1	Strain 3	Strain 4	Strain 8	Strain 9	Strain 10	Strain 12	Strain 13	Azospirullum
Av N(mg/kg)	0 D	233.33±16.16	233.33±16.16	242.66±32.33	233.33±16.16	214.66±16.16	233.33±16.16	224.00±28	233.33±16.16	233.33±16.17	242.66±16.16
	7 D	205.33±16.17	578.66±16.16	616±48.49	578.66±16.16	541.33±16.16	578.66±16.16	569.33±16.17	625.33±16.17	644.00±0.00	532.00±0.00
	14 D	196.00±0.00	242.66±16.16	270.66±16.17	326.66±32.33	261.33±16.17	308.00±0.00	270.66±16.17	298.66±16.16	354.66±16.17	233.33±16.16
	21 D	130.66±32.33	513.33±16.16	616.00±0.00	522.66±16.17	522.66±16.17	550.66±16.16	578.66±16.16	588.00±28	634.66±16.17	186.66±64.66
P(T)= 0.00	28 D	130.66±16.16	644.00±28	578.66±16.16	718.66±42.77	952.00±28	886.66±16.16	896.00±28	877.33±16.17	858.66±32.33	308.00±48.49
LSD(T)= 6.68	35 D	56.00±28	466.66±16.17	578.66±42.78	420.00±28	653.33±16.17	653.33±32.33	606.66±16.17	504.00±28	606.66±16.17	149.33±42.77
Urease((µg/g)	0 D	0.11±0.00	0.12±0.01	0.12±0.00	0.11±0.00	0.12±0.01	0.12±0.01	0.11±0.00	0.11±0.00	0.11±0.00	0.12±0.01
	7 D	0.43±0.02	76.03±0.03	82.86±0.02	78.98±0.01	51.96±0.05	77.28±0.06	73.54±0.02	78.15±0.03	84.95±0.06	18.40±0.00
	14 D	0.43±0.02	60.13±0.02	76.19±0.13	84.95±0.06	66.16±0.13	80.06±0.06	77.21±0.14	78.15±0.05	84.92±0.07	18.59±0.03
	21 D	0.56±0.06	64.72±0.02	89.85±0.01	89.93±0.12	81.71±0.09	91.71±0.06	89.81±0.06	76.11±0.19	92.68±0.11	20.18±0.03
P(T)= 0.00	28 D	24.09±0.60	86.47±0.09	55.93±0.01	82.29±0.02	118.12±0.04	97.59±7.97	107.09±0.05	103.84±0.06	101.33±0.07	28.98±0.06
LSD(T)= 0.26	35 D	24.09±0.60	41.03±0.07	65.50±0.07	60.02±0.40	159.28±0.03	124.65±0.07	111.01±0.05	196.78±0.28	116.31±0.23	51.53±0.09
TKN(%)	0 D	4.15±0.08	4.10±0.08	4.10±0.08	4.06±0.14	4.15±0.16	4.06±0.14	4.10±0.08	4.06±0.14	4.01±0.08	4.10±0.08
	7 D	4.20±0.00	0.51±0.08	0.27±0.00	0.55±0.00	0.65±0.08	0.51±0.08	0.60±0.08	0.46±0.08	0.32±0.08	1.49±0.08
	14 D	3.73±0.08	1.63±0.08	1.45±0.08	1.54±0.14	1.17±0.08	1.12±0.14	1.17±0.08	1.12±0.04	0.89±0.08	2.10±0.14
	21 D	4.06±0.14	0.84±0.14	0.55±0.00	0.65±0.08	0.93±0.16	0.65±0.08	0.60±0.08	0.60±0.08	0.46±0.08	1.21±0.08
P(T)= 0.00	28 D	3.59±0.21	1.30±0.08	1.21±0.16	0.74±0.16	0.56±0.14	0.65±0.08	0.60±0.08	0.84±0.14	0.88±0.16	1.58±0.08
LSD(T)= 0.11	35 D	4.06±0.14	3.36±0.14	2.98±0.16	3.36±0.14	2.47±0.08	2.19±0.08	2.70±0.08	3.03±0.08	2.66±0.14	3.54±0.21

3.14 Field study

As discussed in the earlier section, different combinations of vermicomposted Organic waste (USW) were used as nutritional source in rice. *Sali* (*Kharif*) rice cultivation was undertaken for the study. The basic soil under study was characterized as shown below (Table 14).

Table 14: Basic physicochemical properties of the soil under study

Paramaters	Result
pH	5.3±0.08
Bulk density(g/cc)	1.2±0.02
N(mg/kg)	276±74.08
P(mg/kg)	26.9±0.37
K(mg/kg)	47±0.13
Soil organic C (SOC) (%)	1.6±0.10
Fulvic acid C (FAC) (%)	0.1±0.20
Humic acid C (HAC) (%)	0.1±0.13

Table 15. Changes in soil organic C (TOC), available N, P and K in soil under different treatment combinations during the time of cultivation.

	SOC(%)			N(mgkg-1)			P(mgkg-1)			K(mgkg-1)		
Treatments	t	p	h	t	p	h	t	p	h	t	p	h
T1(NPK100)	1.4±0.18	1.7±0.15	2.1±0.14	470.4±70.4	492.8±56.0	515.2±28.0	38±0.03	39.7±0.02	40.5±1.53	62.2±1.4	71.8±4.2	74.4±5.4
T2(NPK100+FYM)	1.4±0.14	1.5±0.20	2.2±0.14	571.2±28.0	604.8±74.0	616±74.0	52.6±0.03	54±0.15	56.6±4.77	63.0±2.8	72.3±1.7	76.1±6.9
T3(NPK100+VCei)	2.3±0.12	2.5±0.27	3.1±0.07	604.8±42.7	683.2±28.0	694.4±90.0	61.0±0.14	62.5±0.03	63.0±2.18	75.0±3.4	80.4±8.9	98.1±5.6
T4(NPK100+VCmp)	2.2±0.14	2.4±0.27	2.6±0.07	649.6±70.4	660.8±74.0	660.8±48.5	45.5±0.09	58.7±0.04	65.7±1.46	82.3±9.6	87.1±3.4	104.2±3.0
T5(NPK80+VCei)	1.9±0.18	2.5±0.20	3.0±0.14	694.4±56.7	705.6±74.0	705.6±42.7	55±0.03	59.2±0.03	61.1±2.57	88.1±6.2	95.2±2.8	107.2±8.3
T6(NPK80+VCmp)	2.2±0.14	2.4±0.33	2.7±0.07	761.6±100.9	761.6±56.0	772.8±42.7	64.9±0.03	66.2±0.07	67.0±1.21	93.8±2.3	97.6±2.2	119.4±9.2
T7(NPK60+Vcei)	3.2±0.07	3.2±0.22	3.5±0.18	694.4±70.47	705.6±74.0	750.4±42.7	70.4±0.12	71±0.05	71.5±2.0	94.1±1.5	100.8±9.4	128.3±6.1
T8(NPK60+VCmp)	2.7±0.32	2.9±0.27	3.2±0.12	750.4±42.9	772.8±56.0	784±28.0	66.2±0.03	68.5±0.07	71.8±1.98	95.2±7.8	103.8±4.4	130.1±3.6
T9(NPK80+FYM)	2.0±0.18	2.3±0.20	2.4±0.12	560±68.0	593.6±84.0	604.8±58.2	55±0.04	57±0.05	59.5±1.06	75.2±7.6	84.4±2.2	95.74±7.0
T10(NPK60+FYM)	2.3±0.18	2.3±0.20	2.5±0.18	571±53.0	593.6±56.0	616±44.0	57±0.10	59.2±0.88	60.0±1.56	76.1±1.6	83.4±6.8	96.4±4.4
T11(VCei)	2.2±0.14	2.3±0.22	2.5±0.14	571.2±60.9	582.4±74.0	616±42.7	58.3±0.20	59.7±0.11	61.0±1.26	77.9±1.8	89.3±4.4	101.1±9.6
T12(VCmp)	2.0±0.21	2.5±0.20	2.6±0.18	593.6±56.00	604.8±56.0	627.2±56.0	57±0.21	59.9±0.03	61.3±0.93	78.2±0.4	85.4±6.1	108.8±3.6
P value	0.00			0.00			0.00			0.00		
LSD (tr)	0.142			39.849			1.822			4.167		
LSD (yr)	0.124			23.006			1.052			2.405		
LSD (st)	0.121			19.924			0.911			2.083		

Table 15 presents the data on changes in major nutrients and organic carbon in soil during the wet cultivation. Interestingly, N mineralization improved gradually at the end of the cultivation. Significant increase in mineralizable N was recorded under T8 followed by T6 and T7 (P 0.000, LSD 39.84) after cultivation. Addition of vermicompost may increase N-fixing microorganism in soil by many fold and thus have a long term beneficial effect on soil N mineralization (Masciandaro et al., 2000). P availability in soil

gradually increased under all the treatments as compared to the initial value (Table 15). This may be due to enhancement in plant uptake of the nutrient. Similarly K availability of the soil increased by many folds under vermicompost containing treatments was also evidenced from the study. Significant enhancement in K availability was recorded under T7 and T8 followed by T6,T5 and T12.Vermicomposting promotes release of several exogenous and endogenous enzymes as well as improvement in CEC of soils (Sahariah et al., 2015).

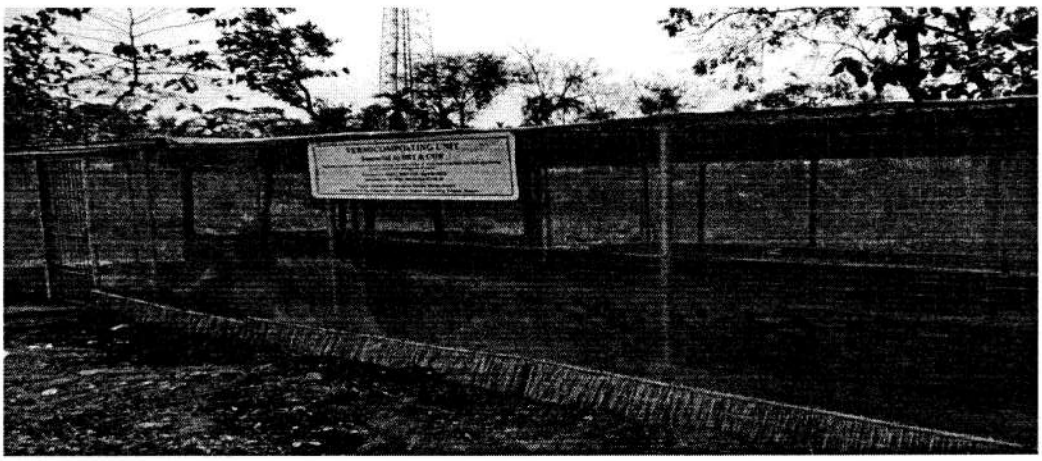
The data on SOC dynamics and its humified fractions during different stages of cultivation is presented in Table 4. SOC significantly increased under vermicompost treatments and maximum gain in SOC was recorded in T7 towards the end of cropping period. Our result is in good agreement with recent findings (Sahariah et al., 2015).

Table 16. Impact of various treatments on Biomass content, Crop Growth Rate (CGR) and Yield(ton ha $^{-1}$) of Sali (kharif) rice

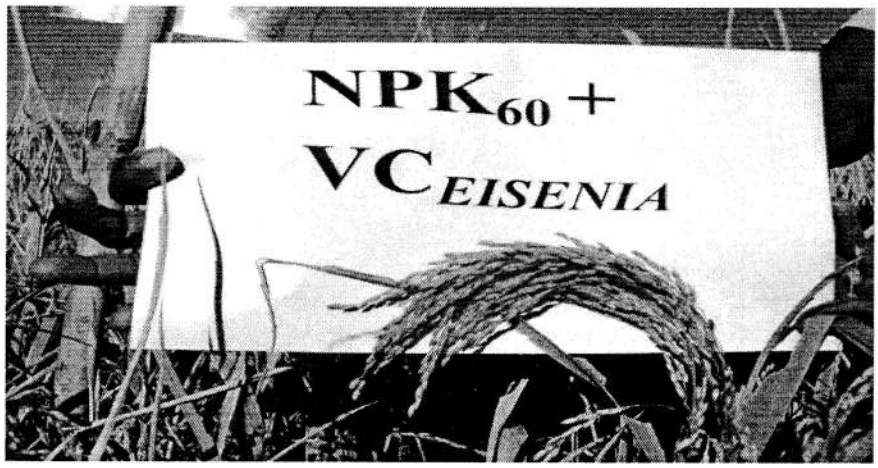
	Biomass content(g/plant)			CGR(g/m2/day)		Yield(tonha^{-1})	
Treatments	t	p	h	p-t	h-p	h	
T1(NPK100)	60.68±2.29	101.64±7.77	116.30±15.28	0.100 ± 0.14	0.064 ± 0.60	6.14 ± 0.83	
T2(NPK100+FYM)	58.27±4.03	98.97±13.32	146.98±14.08	0.099 ± 0.25	0.209 ± 1.16	6.54±0.83	
T3(NPK100+VCei)	64.78±7.99	94.96±5.00	117.84±9.43	0.074 ± 0.20	0.099 ± 0.40	6.65±0.86	
T4(NPK100+VCmp)	57.70±9.20	128.05±17.03	137.27±19.96	0.172 ± 0.52	0.040 ± 0.19	6.46±0.59	
T5(NPK80+VCei)	69.17±8.34	103.87±16.33	128.35±31.66	0.085 ± 0.59	0.106 ± 0.68	6.27±0.13	
T6(NPK80+VCmp)	52.63±1.72	86.53±16.49	118.24±27.14	0.083 ± 0.36	0.138 ± 0.78	6.59±0.38	
T7(NPK60+Vcei)	58.60±7.16	87.72±12.24	134.17±22.75	0.071 ± 0.30	0.202 ±1.52	6.65±0.78	
T8(NPK60+VCmp)	54.37±9.50	95.78±8.80	122.90±17.32	0.101± 0.08	0.118 ±0.41	6.83±0.10	
T9(NPK80+FYM)	53.96±11.11	103.18±9.31	136.12±19.14	0.120 ± 0.40	0.143 ± 0.81	6.60±0.41	
T10(NPK60+FYM)	55.03±4.46	111.30±31.55	136.67±21.07	0.137 ± 0.77	0.110 ± 0.64	6.54±0.17	
T11(VCei)	50.96±1.85	107.38±9.62	133.24±11.12	0.138 ± 0.22	0.112 ±0.14	6.13±0.47	
T12(VCmp)	55.56±11.17	94.26±9.87	149.74±16.84	0.094 ±0.48	0.241 ±0.85	6.05±0.13	
LSD (tr)	3.684			0.133		P value	0.000
LSD (st)	1.842					LSD	0.154

The data on plant biomass content, Crop growth rate (CGR) and grain yield are presented on Table 16. Interestingly, significant biomass yield was recorded under T4,T7,T2,T9 and T10 (P value=0.00;LSD=3.684)..Whereas profuse crop growth rate was recorded under T12 followed by T7,T6,T9 and T5 during the cropping season. However, the cultivation of Sali Rice resulted in highly impressive grain yield (Pvalue=0.00;LSD=0.133).Significantly high grain yield was recorded under T8 followed by T3=T7=T6=T9≥T10=T4>T5≥T1=T11=T12(P value=0.00;LSD=0.154). This indicates the beneficial impact of organic manuring over chemical fertilizers.

Some Photographs



Cultivation of Sali Rice



Publications:

S.S. Bhattacharya, K-H. Kim, A. Ullah, L. Goswami, B. Sahariah, P. Bhattacharyya, S.-B. Cho, O-H Hwang (2016). The effects of composting approaches on the emissions of anthropogenic volatile organic compounds: A comparison between vermicomposting and general aerobic composting. *Environmental Pollution*, 208:600-607. **(IF= 4.143)**

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Appendix-A

Details of Assets acquired wholly or substantially out of Govt. grants Register to be maintained by Grantee Institution

Name of the Sanctioning Authority:		Department of Biotechnology
1.	Sl. No.	2
2.	Name of the Grantee Institution	Tezpur University
3.	No. & Date of sanction order	DBT-NER/Agri/16/2012 dt. 31st October 2012, Serial No. 197
4.	Amount of the sanctioned grant	64.84 Lakhs
5.	Brief purpose of the grant	Development of vermicomposting technology
6.	Whether any condition regarding the right of ownership of Govt. in the Property or other assets acquired out of the grant was incorporated in the grant-in-aid sanction order.	No
*7.	Particulars of assets actually credited or acquired.	Kjeltec N analyzer, Upright deep freezer, BOD incubator, Flame Photometer, Hot Air Oven, pH meter, Vermicomposting unit, Table top centrifuge, UV Spectrophotometer, Binocular Fluorescence Compound Microscope
8.	Value of the assets as on	Rs. 2584790 Lakhs
	Purpose for which utilised at present	N, K and protein estimation, soil estimation, culture, Incubation and preservation of microbes, centrifugation for DNA extraction,Visualization of fluorescent compounds
9.	Encumbered or not	No
10.	Reasons, if encumbered	N/A
11.	Disposed of or not	N/A
12.	Reasons and authority, if any, for Disposal	N/A
14.	Amount realised on disposal	N/A
15.	Remarks	The assets are in good condition

26/1/16

(PROJECT INVESTIGATOR)

16/2/16

(FINANCE OFFICER)

Finance Officer
Tezpur University

(HEAD OF THE INSTITUTE)

Registrar
Tezpur University

* List of equipment purchased indicating the item wise costs may please be provided.

List of equipment purchased indicating the item wise costs
(Till 31-10-15)

1. Kjeltec N analyzer	-	Rs. 643437.00
2. Ultra deep freezer	-	Rs. 441000.00
3. Digital Flame Photometer	–	Rs. 54423.00
4. BOD incubator	-	Rs. 96966.00
5. pH meter	-	Rs. 34050.00
6. Hot Air Oven	-	Rs. 29189.00
7. Vermicomposting unit	-	Rs. 75643.00
8. Table-Top Centrifuge	-	Rs.476000.00
9. UV-Spectrophotometer	-	Rs.449664.00
10.Binocular Fluorescence Compound Microscope	-	Rs. 284418.00
TOTAL		Rs.2584790.00

16/1/16

(PROJECT INVESTIGATOR)

16/2/16

(FINANCE OFFICER)
Finance Office.
Tezpur University

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Registrar
Tezpur University

Appendix-B

Utilisation Certificate

(for the financial year ending 31st October 2015)
(From 01-04-15 to 31-10-15)

(Rs. in Lakhs)

1. Title of the Project/Scheme: **Qualitative development of vermicompost technology through isolation of novel microorganisms and their application in agricultural waste management of Assam**

2. Name of the Organization: Tezpur University

3. Principal Investigator: Dr. Satya Sundar Bhattacharya

4. Deptt. of Biotechnology sanction order No. & date of sanctioning the project: DBT-NER/Agri/16/2012 DT. 31.10.2012

5.	Amount brought forward from the previous financial year quoting DBT letter No. & date in which the authority to carry forward the said amount was given:	Rs. 7,67,983
6.	Amount received from DBT during the financial year (*please give No. and dates of sanction orders showing the amounts paid)*:	Rs. 5,99,000
7.	Other receipts/interest earned, if any, on the DBT grants:	NIL
8.	Total amount that was available for expenditure during the financial year (Sl. Nos. 5,6 and 7):	Rs. 13,66,983
9.	Actual expenditure (excluding commitments) incurred during the financial year (statement of expenditure is enclosed)(01-04-13 to 31-03-14):	Rs. 11,19,554
10.	Unspent balance refunded, if any *(Please give details of cheque No. etc.):*	N/A
11.	Balance amount available at the end of the financial year:	Rs. 2,47,429
12.	Amount allowed to be carried forward to the next financial year vide letter No. & date:	N/A

1. Certified that the amount of Rs. 11,19,554 Lakh mentioned against col. 9 has been utilised on the project/scheme for the purpose for which it was sanctioned and that the balance of Rs. 2,47,429 Lakh will be adjusted towards the grants-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 utilised for the purpose for which it was sanctioned.

Kinds of checks exercised:

1. Cash Book
2. Ledgers
3. Vouchers
4. Bank Statement
5. Any Other

28/1/16

(PROJECT INVESTIGATOR)

16/2/16

(FINANCE OFFICER)
Finance Office
Tezpur University

(HEAD OF THE INSTITUTE)
Registrar
Tezpur University

(To be countersigned by the DBT Officer-in-charge)

Appendix-C

Statement of Expenditure referred to in para 9 of the Utilisation Certificate

Showing grants received the Department of Biotechnology and the expenditure incurred during the period from 1st April 2015 to 31st October 2015.

Item	Unspent balance Carried for ward from previous year	Grants received from DBT during the year	Other receipts/interest earned - if any, on the DBT grants	Total of Col. (2+3+4)	Expenditure (excluding commitments) incurred during the year	Balance (5-6)	Remark
1	2	3	4	5	6	7	8
1.Non-recurring							Rs.3.49 has been re-appropirated to Consumable head
(i) Equipments	2,84,418		NIL	2,84,418	2,84,418	NIL	
2.Recurring							
(i)Human Resource	1,33,429	3,00,000	NIL	4,33,429	1,86,000	2,47,429	
(ii) Consumables	3,49,379	2,00,000	NIL	5,49,379	5,98,516	-49,137	
(iii) Travel	-29	30,000	NIL	29,971	41,039	-11,068	
(iv) Contingency	365	50,000	NIL	50,365	1,228	49,137	
(v)Overheads (if applicable)	421	19,000	NIL	19,421	8,353	11,068	
Total:	7,67,983	5,99,000		13,66,983	11,19,554	2,47,429	

28/1/16

(PROJECT INVESTIGATOR)

26/2/16

(FINANCE OFFICER)
Finance Officer
Tezpur University

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Registrar
Tezpur University

Annexure A

Manpower Staffing Details (In the financial year wise manner)

NAME OF THE PERSON	NAME OF THE POST	DATE OF JOINING	DATE OF LEAVING	TOTAL MONTHLY SALARY	TOTAL SALARY PAID DURING THE FINANCIAL YEAR	TOTAL SALARY PAID DURING PROJECT PERIOD
Banashree Sahariah	JRF till January 2015 & SRF from Feb. to Oct. 2015	16-02-13	31.10.2015	14000	102000	390000
Nazneen Hussain	JRF	11-11-13	31.10.2015	12000	84000	272000
					186000	6,62,000

28/1/16

(Signature of Principal Investigator)

16/2/16

(Signature of Accounts Officer)
Finance Officer
Tezpur University

(SIGNATURE OF HEAD OF THE INSTITUTE)

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Annexure B

Manpower Expenditure Details (In financial year wise manner)*:

SANCTIONED POSTS	NUMBER	SCALE OF PAY	ANNUAL OUTLAY	OUTLAY FOR THE ENTIRE PERIOD	REVISED SCALE, IF ANY	REVISED ANNUAL OUTLAY	REVISED PROJECT OUTLAY	ACTUAL RELASES BY DBT	ACTUAL EXPENDITURE	BALANCE
JRF (One JRF has been promoted to SRF w.e.f Feb 2015)	2	12000 & 14000 for one SRF w.e.f Feb 2015	3.00 Lacs	13.20 Lacs	-	-	-	3.00 lacs + Last year bal. of Rs. 1,33,429 Lacs = **4.33429 lacs**	1.86 Lacs	2.47429 Lacs

28/1/16

(Signature of Principal Investigator)

16-2-16

(Signature of Accounts Officer)

Finance Officer
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* Details of manpower salary/fellowship revision along with due-drawn statement and arrears requested should be given separately, if applicable.

Due- Drawn Statement

Name of the Project Staff	Month and Year	Due	Drawn	Difference
Banashree Sahariah	Apr, 2015 May, 2015 June, 2015 July, 2015 Aug, 2015 Sep, 2015 Oct, 2015	Rs. 98,000 + Rs. 4000 (Arrear for Feb and Mar,2015) = Rs. 102000	Rs. 102000	Nil
Nazneen Hussain	Apr, 2015 May, 2015 June, 2015 July, 2015 Aug, 2015 Sep, 2015 Oct, 2015	Rs. 84,000	Rs. 84,000	Nil

28/1/16

(Signature of Principal Investigator)

16.2.16

(Signature of Accounts Officer)
Finance Officer
Tezpur University

(SIGNATURE OF HEAD OF THE INSTITUTE)
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Appendix-B

Utilisation Certificate

(for the financial year ending 31st October 2015)
(From 01-04-15 to 31-10-15)

(Rs. in Lakhs)

1. Title of the Project/Scheme: **Qualitative development of vermicompost technology through isolation of novel microorganisms and their application in agricultural waste management of Assam**

2. Name of the Organization: Tezpur University

3. Principal Investigator: Dr. Satya Sundar Bhattacharya

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9.	Actual expenditure (excluding commitments) incurred during the financial year (statement of expenditure is enclosed)(01-04-13 to 31-03-14):	Rs. 11,19,554
10.	Unspent balance refunded, if any *(Please give details of cheque No. etc.):*	N/A
11.	Balance amount available at the end of the financial year:	Rs. 2,47,429
12.	Amount allowed to be carried forward to the next financial year vide letter No. & date:	N/A

1. Certified that the amount of Rs. 11,19,554 Lakh mentioned against col. 9 has been utilised on the project/scheme for the purpose for which it was sanctioned and that the balance of Rs. 2,47,429 Lakh will be adjusted towards the grants-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 utilised for the purpose for which it was sanctioned.

Kinds of checks exercised:

1. Cash Book
2. Ledgers
3. Vouchers
4. Bank Statement
5. Any Other

28/1/16

(PROJECT INVESTIGATOR)

16.2.16

(FINANCE OFFICER)

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(To be countersigned by the DBT Officer-in-charge)

Appendix-C

Statement of Expenditure referred to in para 9 of the Utilisation Certificate

Showing grants received the Department of Biotechnology and the expenditure incurred during the period from 1st April 2015 to 31st October 2015.

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(i)Human Resource	1,33,429	3,00,000	NIL	4,33,429	1,86,000	2,47,429	
(ii) Consumables	3,49,379	2,00,000	NIL	5,49,379	5,98,516	-49,137	
(iii) Travel	-29	30,000	NIL	29,971	41,039	-11,068	
(iv) Contingency	365	50,000	NIL	50,365	1,228	49,137	
(v)Overheads (if applicable)	421	19,000	NIL	19,421	8,353	11,068	
Total:	7,67,983	5,99,000		13,66,983	11,19,554	2,47,429	

(PROJECT INVESTIGATOR)

16-2-16

(FINANCE OFFICER)

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