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Eco-Friendly Treatment of Biomedical Waste Using *Perionyx excavatus*

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Selvalakshmi M, Greeshma G, *Pawlin Vasanthi Joseph

Department of Zoology, Nirmala College for Women (Autonomous), Coimbatore-641018, Tamilnadu, India

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ABSTRACT

Vermicomposting is a process of production of compost by breeding earthworms, resulting in homogeneous and stabilized humus used as manure. Worms in the process of feeding on waste cause bio-oxidation by relentless turning, fragmentation and aeration of waste by devouring resulting in homogeneous and stabilized humus like product. Biomedical waste, also known as infectious waste or medical waste is defined as solid waste generated during the diagnosis, testing, treatment, research or production of biological products for humans or animals. Four tones of Medical waste from 400 healthcare institutions in Coimbatore and Tirupur districts are processed every day by Tekno Therm Industries, which operates the TNPCB- authorized biomedical waste treatment facility in Coimbatore. The aim of the present work is to employ *Perionyx excavatus* for conversion of biomedical waste from hospitals into useable manure. To each of the experimental pots maintained for vermicomposting, 3kg of primarily decomposed bio-medical waste (previously prepared) was added. *Perionyx excavatus* were released into the pots. Analysis was carried out after every 15 days for a total period of 60 days. The biological parameters such as individual adult worm weight, length of individual worm, number of cocoons, juveniles and adult worms and worm biomass has significantly increased. Temperature has significantly decreased. pH has come to neutral. The Nitrogen (N), Phosphorus (P), Potassium (K) has significantly increased. The microbial content of *E. coli, Staphylococcus aureus, Klebsiella pneumoniae, Bacillus cereus, and Bacillus subtilis* has significantly decreased making the vermicompost a rich manure.

INTRODUCTION

Vermicomposting is the breaking down of organic material through the use of worms, bacteria, and fungi. In nature, organic matter is decomposed through these organisms. By managing vermicomposting you are essentially speeding up Mother Nature's process of breaking down organic matter. The end product of vermicompost or "worm castings" is a nutrient rich organic substance that can be added to soil to increase its organic matter content and available nutrients.

Vermicomposting is a process of production of compost by breeding earthworms, resulting in homogeneous and stabilized humus used as manure. Worms in the process of feeding on waste cause bio-oxidation by relentless turning, fragmentation and aeration of waste by devouring resulting in homogeneous and stabilized humus like product. The fine granular peat-like end product, vermicompost that is produced contains elevated levels of nitrogen, phosphorus, and potassium (NPK) in available form, micronutrients, microflora, enzymes, and growth regulators. (Mathur *et al.,* 2006).

Earthworms are voracious feeders of organic wastes and they utilize only a small portion of these wastes for their growth and excrete a large proportion of wastes consumed in a half digestion form (Jambhekar 1992). Earthworm's intestine contains a wide range of microorganisms, enzymes and hormones which aid rapid decomposition of half- digested material transforming them into vermicompost in a short time (nearly 4-8 weeks) (Nagavallemma *et al.,* 2004). As the organic matter passes through the gizzard of the earthworm it is grounded into a fine powder after which the digestive enzymes, microorganisms and other fermenting substances act on them further aiding their breakdown within the gut, and finally passes out in the form of "casts" which are later acted upon by earthworm gut associated microbes converting them into mature product, the "vermicompost".

Over the last few years, the problem of efficient disposal and management of organic solid wastes has become more rigorous due to rapidly increasing population, intensive agriculture, and industrialization (Garg *et al.,* 2006). Production of large quantities of organic wastes all over the world poses major environmental (offensive odors, contamination of groundwater and soil) and disposal problems (Edwards and Bater, 1992). Appropriate disposal of waste is most essential and beneficial from ecological and economical point of view. Although there

are many ways of organic waste treatment, composting is one of the best acceptable ways for quality environment and organic farming.

Biomedical waste, also known as infectious waste or medical waste is defined as solid waste generated during the diagnosis, testing, treatment, research or production of biological products for humans or animals. Biomedical waste includes syringes, live vaccines, laboratory samples, body parts, bodily fluids and waste, sharp needles, cultures and lancets. Biomedical waste is any kind of waste containing infectious materials. It may also include waste associated with the generation of biomedical waste that visually appears to be of medical or laboratory origin.

WHO stated that 85% of hospital wastes are actually non-hazardous, around 10% are infectious and around 5% are non-infectious but hazardous wastes. In India, this could range from 15% to 35% depending on the total amount of waste generated (Glenn and Garwal, 1999; Chitnis *et al.*, 2005). Four tones of Medical waste from 400 healthcare institutions in Coimbatore and Tirupur districts are processed every day by TeknoTherm Industries, which operates the TNPCB- authorized biomedical waste treatment facility in Coimbatore. According to S. Sudhakar, partner to TeknoTherm Industries, the firm invested around Rs.5 crore to set up the facility near chettipalayam, around 30km from the city, in 2005 and is run in collaboration with the Indian Medical Association.

The aim of the present work is to employ *Perionyx excavatus* for conversion of biomedical waste from hospitals into useable manure. The study involved the estimation of the biological parameters of earthworms in the biomedical waste, analyzed the physical and nutrient parameters of the vermicompost and assessed the depletion rate of pathogenic microorganisms in the vermicompost.

MATERIALS AND METHODS

Three replicates were maintained for each of the treatments. Methodology for one of the replicates has been detailed below. The entire study was carried out for a period of 60 days.

Collection of bio-medical waste:

Bio-medical waste (3 kg) was collected from Surya Hospital, Dharapuram, and was used as and when required for the experimentation. For each of the cycle of vermicomposting only

the biodegradable matter of the infected Biomedical waste was considered for the experiment. This included blood stained cotton pieces, pus and body fluids, antiseptics/ antibiotics used for dressing of wounds, spilled liquid and tissues collected from operation tables (Srivatsava *et al.*, 2000).

Preliminary on-site treatment of BioMedical Waste:

The Bio-medical waste used for the experiment was chemically sterilized on-site using 5% of 1N NaOCL. This was done to disinfect the Bio-medical waste before subjecting it to vermicomposting and natural composting (Hugo and Russell, 1992).

Primary decomposition of disinfected Bio-Medical Waste:

Following the chemical treatment, the disinfected Bio-Medical Waste was made palatable or more suitable for the earthworm species to feed. Primary decomposition of Bio-medical waste was carried out in the laboratory for a period of 15 days as follows:

Preparation of cow dung slurry:

A homogenous mixture of cow dung slurry was prepared at 1:4 ratio by mixing 250g of cow dung with 1 litre of distilled water. Three litres of the slurry was prepared and maintained in three separate containers.

Mixing of Bio-Medical Waste with cow dung slurry:

To each of the three containers with 1L cow dung slurry, 1kg of Bio-medical waste was added and mixed. The mixture was allowed to undergo primary decomposition for a period of 15 days in the laboratory. The same procedure was carried out to prepare the control tank. It was also done to facilitate the consumption of Bio-Medical Waste by epigeic earthworms during the process of vermicomposting (Kaushik and Garg, 2003).

Tank preparation:

Preparation of tanks for vermicomposting and natural composting:

Three plastic tanks were maintained to carry out the process of vermicomposting. Each tank used for the experiment measured 1m long, 0.5 m broad and 0.5 m deep. A tank containing 1kg primary decomposed Bio-medical waste but without introduction of any earthworm

species was maintained as control and allowed to undergo natural composting for a period of 60 days. All the tanks were maintained in triplicates.

Collection of suitable epigeic earthworms:

The epigeic earthworm species namely *Perionyx excavatus* was used for the study. They were collected from the vermicompost unit of the Department of Zoology, Nirmala College for Women, Coimbatore.

Release of earthworms into tanks:

To each of experimental pots maintained for vermicomposting 3 kg of primarily decomposed bio-medical waste (previously prepared) was added. *Perionyx excavatus* were released into the pots. Analysis was carried out after every 15 days for a total period of 60 days.

METHODOLOGY

Biological Parameters (Dinesh *et al.*, 2010)

The total number of earthworms was counted after carefully removing the worms manually from the treatment pots. The earthworms removed were rinsed with distilled water to remove all extraneous material, briefly drained on a tissue paper and weighed on a scale (Amitha and Joseph, 2017).

After the completion of 15 days of vermicomposting, average individual adult worm weight, average individual length, number of adult worms, number of cocoons, number of juveniles and total worm biomass was estimated.

Physico- Chemical Parameters

pH – Digital PH meter

Moisture content – Karl Fischer Titration (1935)

Temperature – Thermometric method

Nutrient Analysis

Nitrogen – Kjeldahl method (1883)

Phosphorus – Bray's method (1945)

Potassium – Tetraphenylborate method (1956)

Microbial Study (Pelczar *et al.,* 1986; Swanson *et al.,* 1992).

Isolation of E.coli:

Samples (0.5 ml) were taken in 10 ml LB (Luria Bertani) broth medium in test tube, and vortexed for one minute and left for thirty minutes at room temperature. Then supernatant (1 ml) was taken from this test tube and a 2-fold serial dilution was prepared. After this, 500 ml from the final dilution tube was spread on the petri dishes (Pyrex) of MacConkey medium and LB medium. Petri dishes were kept in the incubator for 24 hours at 37°C. After 24 hours, plates were studied for the colonies of microbes grown on the media.

Isolation of *Bacillus cereus:*

A soil suspension (10g / 100ml) was pasteurized, diluted (1:100) and a 0.1 ml sample was spread on MEP (mannitol; egg yolk; polymyxin) agar. MEP agar selects for the growth of polymyxin resistant organisms; *Bacillus cereus* colonies can be differentiated from colonies of the other polymyxin resistant organisms growing on the plate by their inability to ferment mannitol and the presence of lecithinase. *B. cereus* colonies appear pink.

Klebsiella pneumonia:

Samples soil were taken aseptically and plated directly on Tryptic Soya Agar (TSA) (Hi-media). The plates were incubated for 24h at 37°C for the bacterial colonies to appear. Dominate colonies from Tryptic Soya Agar were selected and streaked again on Tryptic Soya Agar plate and also on different selective media plates to obtain pure culture. The pure culture of *K. pneumonia* (BB12) was grown in Tryptic Soya Broth (TSB) and maintained as the glycerol stock at-20°C.

Bacillus subtillus:

Serially dilute the sample and spread it on nutrient agar or LB agar. Pasteurize the sample (80°C for 15-30 min) before spreading. Most of the times *B. subtillus* swarm on low % agar plates, so it is recommended using a 2% agar.

Staphylococcus aureus:

Mannitol Salt Agar (MSA) is used as a selective and differential medium for the isolation and identification of *Staphylococcus aureus*. It encourages the growth of a group of certain bacteria while inhibiting the growth of others.

RESULTS AND DISCUSSION

Table 1: Growth Parameters of earthworm in Bio-medical waste

Earthworm Growth Parameters	15 Day	30 Day	45 Day	60 Day
Individual Adult Worm Weight (g)	0.87±0.15	1.13±0.15	1.33±0.21	1.70±0.20
Individual Length (cm)	6.77±0.15	7.53±0.21	8.03±0.15	8.83±0.21
No. of Adult Worms	4.00±2.00	7.00±1.00	8.00±3.00	32.33±2.52
No. of Cocoons	12.00±2.00	28.33±2.52	42.00±3.00	53.33±3.06
No. of Juveniles	25.67±3.51	39.00±4.00	47.00±3.00	55.67±3.51
Total Worm Biomas (g)	3.47±0.35	4.67±0.42	5.14±0.03	6.25±0.04

Values are Mean ± Standard Deviation of the samples in each group.

Table 2: Temperature of vermicompost during experimental period

SAMPLE	DAYS OF TREATMENT			
	15	30	45	60
CONTROL	31.70±0.20**	31.60±0.20**	31.70±0.20**	31.63±0.15*
EXPERIMENT	29.63±0.37**	27.13±1.43**	26.63±0.97**	25.33±2.51*

Values are Mean ± Standard Deviation of the sample in each group; ***** - Significant at $p< 0.05$ level; ****** - Significant at $p< 0.01$ level.

Table 3: pH of vermicompost during experimental period

SAMPLE	DAYS OF TREATMENT			
	15	30	45	60
CONTROL	8.10±0.20*	7.83±0.30**	7.70±0.20**	7.60±0.10*
EXPERIMENT	6.0±1.0*	6.53±0.30**	6.90±6.20**	7.16±0.25*

Values are Mean ± Standard Deviation of the sample in each group; ***** - Significant at p< 0.05 level; ****** - Significant at p< 0.01 level.

Table 4: Moisture content of vermicompost during experimental period

SAMPLE	DAYS OF TREATMENT			
	15	30	45	60
CONTROL	65.30±0.95**	66.53±0.58**	66.80±1.05*	70.40±0.79*
EXPERIMENT	53.0±1.0**	61.83±1.23**	66.30±2.0*	69.36±1.02*

Values are Mean ± Standard Deviation of the sample in each group; ***** - Significant at p< 0.05 level; ****** - Significant at p< 0.01 level.

Table 5: Nitrogen content of vermicompost during experimental period

SAMPLE	DAYS OF TREATMENT	
	30	60
CONTROL	0.24±0.04**	0.30±0.02**
EXPERIMENT	0.45±0.04**	0.69±0.04**

Values are Mean ± Standard Deviation of the sample in each group; ***** - Significant at p< 0.05 level; ****** - Significant at p< 0.01 level.

Table 6: Phosphorus content of vermicompost during experimental period

SAMPLE	DAYS OF TREATMENT	
	30	60
CONTROL	0.34 ± 0.39^{NS}	0.14±0.03**
EXPERIMENT	0.15 ± 0.04^{NS}	0.33±0.03**

Values are Mean ± Standard Deviation of the sample in each group; ***** - Significant at p< 0.05 level; ****** - Significant at p< 0.01 level.

Table 7: Potassium content of vermicomposting during experimental period

SAMPLE	DAYS OF TREATMENT	
	30	60
CONTROL	0.11±0.01*	0.15±0.03**
EXPERIMENT	0.20±0.04*	0.35±0.02**

Values are Mean ± Standard Deviation of the sample in each group; ***** - Significant at p< 0.05 level; ****** - Significant at p< 0.01 level.

Table 8: Microbial content of vermicompost during experimental period

Pathogens	SAMPLE	DAYS OF TREATMENT		
		1	30	60
E. coli	CONTROL	6.0±0.20**	5.33±0.55*	4.50±0.40**
	EXPERIMENT	5.30±0.20**	4.20±0.25*	3.20±0.30**
Staphylococcus aureus	CONTROL	8.0±0.10NS	6.20±0.30**	4.10±0.30**
	EXPERIMENT	5.10±0.20NS	3.83±0.30**	1.76±0.15**
Kblebsiella pneumonia	CONTROL	7.33±0.15**	5.13±0.35**	3.33±0.55*
	EXPERIMENT	4.60±0.30**	4.0±0.20**	2.66±0.41*
Bacillus cereus	CONTROL	7.03±0.15NS	5.10±0.36**	3.10±0.40*
	EXPERIMENT	4.03±0.15NS	3.0±0.50**	2.50±0.40*
Bacillus subtillus	CONTROL	5.46±0.25**	4.53±0.30**	3.43±0.50*
	EXPERIMENT	3.0±0.20**	2.56±0.55**	2.03±0.25*

Values are Mean ± Standard Deviation of the sample in each group; ***** - Significant at p< 0.05 level; ****** - Significant at p< 0.01 level.

Table 9: One way ANOVA for Physicochemical Parameters analyzed during the experimental period

PARAMETERS	DAYS	Df	SS	MS	F	P	CV %
TEMPERATURE	15	3	6.406667	6.406667	295.6923	0.000**	0.48
	30		29.926667	29.926667	38.2857	0.025*	3.01
	45		38.506667	38.506667	127.6464	0.000**	1.88
	60		59.535000	59.535000	21.3005	0.044*	5.87
pH	15	3	6.615000	6.615000	20.6719	0.045*	8.02
	30		2.535000	2.535000	2655.0291	0.000**	0.00
	45		0.806667	0.806667	17.2857	0.053*	2.95
	60		0.281667	0.281667	24.1429	0.039*	1.46
MOISTURE CONTENT	15	3	226.935000	226.935000	45387.0000	0.000**	0.12
	30		13.801667	13.801667	36.1616	0.027*	0.95
	45		6.201667	6.201667	195.8421	0.000**	0.27
	60		4.506667	4.506667	96.5714	0.010**	0.31

df– degrees of freedom; **SS** – Sum of squares; **MS** – Mean Square; **F** – F-test; **P** – Probability; **CV** – Coefficient of Variation; ****** - Significant at P < 0.01 level; *****- Significant at P < 0.05 level.

Table 10: One way ANOVA for Nutrient Parameters analyzed during the experimental period

PARAMETERS	DAYS	Df	SS	MS	F	P	CV%
NITROGEN	**30**	5	0.062017	0.062017	3721.0000	0.000**	1.17
	60		0.232067	0.232067	1071.0769	0.000**	2.94
PHOSPHORUS	**30**	5	0.004817	0.004817	22.2308	0.042*	11.47
	60		0.052267	0.052267	3136.0000	0.000**	1.72
POTASSIUM	**30**	5	0.010417	0.010417	32.8947	0.029*	11.24
	60		0.060000	0.060000	1200.0000	0.000**	2.83

df– degrees of freedom; **SS** – Sum of squares; **MS** – Mean Square; **F** – F-test; **P** – Probability; **CV** – Coefficient of Variation; ****** - Significant at P < 0.01 level; *****- Significant at P < 0.05 level.

Table 11: One way ANOVA for Microbial Parameters analyzed during the experimental period

PATHOGENS	DAYS	Df	SS	MS	F	P	CV%
E.coli	**1**	8	0.735000	0.735000	5172.1027	0.000**	0.00
	30		1.706667	1.706667	36.5714	0.026*	4.50
	60		2.535000	2.535000	507.0000	0.000**	1.84
Staphylococcus aureus	**1**	8	12.615000	12.615000	2523.0000	0.000**	1.08
	30		8.401667	8.401667	5041.0000	0.000**	0.81
	60		8.166667	8.166667	700.0000	0.000**	3.68
Klebsiella pneumoniae	**1**	8	11.206667	11.206667	960.5714	0.000**	1.81
	30		1.926667	1.926667	165.1429	0.000**	2.37
	60		0.666667	0.666667	30.7692	0.031*	4.91
Bacillus cereus	**1**	8	13.500000	13.500000	300.0000	0.000**	3.83
	30		6.615000	6.615000	441.0000	0.000**	3.02
	60		1.401667	1.401667	64.6923	0.015*	4.99
Bacillus subtilis	**1**	8	1.081667	1.081667	45.0000	0.012*	1.05
	30		5.801667	5.801667	183.2105	0.000**	5.01
	60		2.940000	2.940000	84.0000	0.012*	6.84

df– degrees of freedom; **SS** – Sum of squares; **MS** – Mean Square; **F** – F-test; **P** – Probability; **CV** – Coefficient of Variation; ****** - Significant at $P < 0.01$ level; *****- Significant at $P < 0.05$ level.

Biological parameters:

Individual adult worm weight:

The individual adult worm weight on 15^{th} day is 0.87±0.15, 30^{th} day 1.13±0.15, 45^{th} day 1.33±0.21 and 60^{th} day 1.70±0.20.

Individual length:

The individual adult worm length on 15^{th} day is 6.77±0.15, 30^{th} day 8.03±0.15, 45^{th} day 8.03±0.15 and 60^{th} day 8.83±0.21.

Number of adult worms:

The Number of adult worms on 15th day is 4.00±2.00, 30th day 7.00±1.00, 45th day 18.00±3.00 and 60th day 32.33±2.52.

Number of cocoons:

The number of cocoons on the 15th day is 12.00±2.00, 30th day 28.33±2.52, 45th day

42.00±3.00 and 60th day 53.33±3.06.

Number of juveniles:

The number of juveniles on the 15th day is 25.67±3.51, 30th day 39.00±4.00, 45th day

47.00±3.00 and 60th day 55.67±3.51.

Total worm biomass:

The total worm Biomass on the 15th day is 3.47±0.35, 30th day 4.67±0.42, 45th day 5.14±0.03 and 60th day 6.25±0.04.

Physico-chemical parameters:

Temperature:

The temperature of the control for the 15thday is 31.70±0.20, 30th day 31.60±0.20, 45th day 31.70±0.20 and 60th day 31.63±0.15, ($p < 0.05$). The experimental value on the 15th day has decreased to 29.63±0.37, 30th day 27.13±1.43, 45th day 26.63±0.97 and 60th day 25.33±2.51, ($P < 0.05$). The one way ANOVA for temperature is significant at 5% level.

pH:

The pH of the control for the 15th day is 8.10±0.20, 30th day 7.83±0.30, 45th day 7.70±0.20 and 60th day 7.60±0.10, ($P < 0.05$). The experimental value on the 15th day has increased to 6.0±1.0, 30th day 6.53±0.30, 45th day 6.90±0.20 and 60th day 7.16±0.25, ($P < 0.05$). The one way ANOVA for pH is significant at 5% level.

Moisture content:

The Moisture content of the control for the 15th day is 65.30±0.95, 30th day 66.53±0.58, 45th day 66.80±1.05 and 60 days 70.40±0.79, (P < 0.05). The experimental value on the 15th day has increased to 53.0±1.0 and 30th day 61.83±1.23 and 45th day 66.30±2.0 and 60th day 69.36±1.02, (P < 0.05). The one way ANOVA for Moisture content is significant at 5% level.

Total Nitrogen (N):

The total nitrogen (N) content of the control for the 30thday is 0.24±0.04 and 60th day 0.30±0.02, (P < 0.01). The experimental value on the 30th day has increased to 0.45±0.04 and 60th day 0.69±0.04 (P <0.01). The one way ANOVA for nitrogen is significant at 1% level.

Phosphorus (P):

The Phosphorus (P) content of the control for the 30th day is 0.34±0.39 and 60th day 0.14±0.03, (P < 0.01). The experimental value on the 30th day has increased to 0.15±0.04 and 60th day 0.33±0.03, (P <0.01). The one way ANOVA for phosphorus is significant at 1% level.

Potassium (K):

The Potassium (K) content of the control for the 30th day is 0.11±0.01 and 60th day 0.15±0.03, (P <0.01). The one way ANOVA for potassium is significant at 1% level.

Microbial analysis:

Escherichia coli:

The *E. coli* control on the 1st day is 6.0±0.20, 30th day 5.33±0.55 and 60th day 4.50±0.40, (P <0.01). The experimental value on the 1st day has decreased to 5.30±0.20, 30th day 4.20±0.25 and 60th day 3.20±0.30, (P <0.01). The one way ANOVA for E. *coli* is significant at 1% level.

Staphylococcus aureus:

The *Staphylococcus aureus* control on the 1st day is 8.0±0.10, 30th day 6.20±0.30 and 60th day 4.10±0.30, (P <0.01). The experimental value on the 1st day has decreased to 5.10±0.20, 30th

day 3.83±0.30 and 60th day 1.76±0.15, (P <0.01). The one way ANOVA for *Staphylococcus aureus* is significant at 1%level.

Klebsiella pneumoniae:

The *Klebsiella pneumoniae* control on the 1st day is 7.33±0.15, 30th day 5.13±0.35 and 60th day 3.33±0.55, (P <0.05). The experimental value on the 1st day has decreased to 4.60±0.30, 30th day 4.0±0.20 and 60th day 2.66±0.41, (P <0.05). The one way ANOVA for *Klebsiella pneumoniae* is significant at 5% level.

Bacillus cereus:

The *Bacillus cereus* control on the 1st day is 7.03±0.15, 30th day 5.10±0.36 and 60th day 3.10±6.40, (P <0.05). The experimental value on the 1st day has decreased to 4.03±0.15, 30th day 3.0±0.50 and 60th day 2.50±0.40, (P <0.05). The one way ANOVA for *Bacillus cereus* is significant at 5% level.

Bacillus subtilis:

The *Bacillus subtilis* control for the 1st day is 5.46±0.25, 30th day 4.53±0.80 and 60th day 3.43±0.50, (P<0.05). The experimental value on the 1st day has decreased to 3.0±0.20, 30th day 2.56±0.55 and 60th day 2.03±0.25, (P <0.05). The one way ANOVA for *Bacillus subtilis* is significant at 5% level.

Biological parameters:

Suthar and Singh, 2008 reported that the worms when introduced into wastes showed an increased growth rate and reproductive activities.The growth rate has been considered as a good comparative index to compare the growth of earthworms in different waste (Edwards *et al*.,1998).The factor that influences the number of worms is directly related to the cocoon production and number of earthworms. As the number of cocoons increases, there is increase in the number of worms due to the hatchlings from the cocoons (Manaf *et al*., 2009). A favorable environment will also increase the number of worms with less or no mortality.

The difference between rates of cocoon production could be related to the biochemical quality of the feeds, which is important in determining the time taken to reach sexual maturity and onset of reproduction (Flack and Hartenstein, 1985). Feeds which provide earthworms

with sufficient amount of easily metabolizing organic matter and non-assimilated carbohydrates favor growth and reproduction of earthworms.

Recently it has been reported that along with feed quality the microbial biomass and decomposition activities are also important (Suthar, 2005). The results indicated that the quality and palatability of the substrate directly affect the survival, growth rate and reproduction potential of earthworms (Tripathi and Bhardwaj, 2004a; Gajalakshmi*et al.*, 2005).

The earthworm biomass gain is directly related to the feeding rate, palatability of feedstuff and particle size of feedstock; however, there is a close relationship between feedstock quality and microbial richness of bedding substrates which directly or indirectly affects the earthworm feeding rate, as microbes are the important component of earthworm diet. Additionally, the earthworms may have utilized microorganisms present in their substrates as food source and could digest them selectively (Suthar, 2009; Singh and Sharma, 2002). The readily available nutrients in the substrate would also enhance the feeding activity of the worms, showing their increase in biomass (Suthar and Singh, 2008).

The readily available nutrients in the substrate would also enhance the feeding activity of the worms, showing their increase in biomass.(Amitha and Joseph, 2017).

Physico-chemical parameters:

Suthar (2010a) reported that the earthworm productivity was high when the temperature and vermibed characteristics were at optimum level. Hait and Tare (2011) reported that environmental temperature, (10-30˚c) was an ideal temperature to activate metabolic activity and induce maximum reproduction action.

The pH values of composting mixtures increased from acidic to neutral. This increase was possibly due to the decomposition of nitrogenous matter excreted by earthworms. Similar observations were made by (Muthukumaravel *et al.*, 2008; Amitha and Joseph, 2017).

The moisture content increased during the thermophilic phase of composting (Larney and Blackshow, 2003). According Liang *et al.*, (2003) the increase in moisture content might be due to the high absorption indicating the higher rate of degradation of waste by earthworms.

Nutrient Parameters

The increase trend in nitrogen in the vermibeds was reported by Curry *et al.,* (1995), Ramalingam (1997). Of the total nitrogen excreted by worms, about half is secreted as mucoproteins by gland cells found in the epidermis, and half in the form of ammonia. Urea and possibly uric acid as allantonin in a fluid excreted from the nephridiopores (Edwards and Lofty, 1977). Graff (1981) reported that the excreta of earthworms had more nitrogen considerably in casts than the surrounding soil. A net mineralization of nitrogen occurred due to earthworm activity, whereas a net loss of mineral nitrogen was observed on exclusion of earthworms (Blair *et al.,* 1995). Amita and Joseph 2017

Earthworms also have a great impact on nitrogen transformations, by enhancing nitrogen mineralization, so that mineral nitrogen may be retained in the nitrate form (Edwards *et al.,* 2000).

The higher population of Phosphate-solubilizers (Chowdappa*et al.,* 1999) or phosphorus is a result of bacterial and faecal phosphate activity of earthworms (Garg*et al.,* 2006). The worms during vermicomposting convert the insoluble P into soluble forms with the help of phosphate-solubilizing microorganisms through phosphatases present in the earthworm gut, making it more available to plants (Suthar and Singh, 2008).

This finding is also supported by Padmavathiamma (2008) who suggested that the status of P content in vermicompost depends on acid formation during organic matter decomposition process by the microorganisms and is the major mechanism for solubilisation of insoluble phosphorus. Le Bayon and Binet (2006) have reported that some amount of phosphorus is converted to more available forms partly by earthworm gut enzymes, i.e. acid phosphatases and alkaline phosphatases. Kale *et al.,* (1982) revealed that the presence of large number of micro flora in the gut of earthworm might have played an important role in increasing P content in the process of vermicomposting.

Vermicomposting has been established as an effective process for recovering higher K from organic waste (Manna *et al.,* 2003; Suthar, 2007). The generation of acid during decomposition of organic matter by the microorganisms is the crucial process for solubilization of insoluble potassium (Adi and Noor, 2009). This result is also found similar to the findings reported by Rao *et al.,* (1996) who suggested that the increase in K of the vermicompost in relation to that of the compost was probably because of physical

decomposition of organic matter of waste due to biological grinding during passage through the gut, coupled with enzymatic activity in worm's gut.

Results are well supported by Kaviraj and Sharma (2003) who stated that the microorganisms present in the worm's gut probably converted insoluble K into the soluble form by producing microbial enzymes. Suthar (2007) in his study also noticed that earthworm processed waste material contains high concentration of exchangeable potassium due to enhanced microbial activity during vermicomposting process which consequently enhanced the rate of mineralization.

Microbial Analysis:

Results of the current study showed that earthworms have a high ability to remove the pathogens with no need of temperature increase in vermicomposting. Decrease of pathogens in vermicompost can perhaps be explained in two ways; first, because it is a part of the earthworm's food, second, removal of pathogens by proteolytic enzymatic activity. The vermicompost has the ability to remove the pathogens considerably. Decrease of the pathogens in vermicomposting depends on different factors such as the enzymatic activity of the earthworm gut, secretion of the coelomic fluids with antibacterial properties, and also competition among different groups of microorganisms.

Rodriguez investigated the reduction of pathogen number in the septic sludge during vermicomposting and showed that the pathogen's number have decreased considerably which is in the same line with results of the present study (Rodriguez *et al.*, 2010). Nair *et al.*, (2006), studied that vermicomposting leads to greater reduction of pathogens after three months upon storage.

It is assumed that the temperature will no longer increase during vermicomposting. In a study investigating the influence of temperature on pathogen content in kitchen waste, it was found that the optimum period to obtain pathogen safety was 9 days of pre-composting, followed by 2.5 months of vermicomposting. This result showed that if pre-conposting, process did not reach a high enough temperature, it was possible that not only the pathogens may be sufficiently inactivated, but also that they would even proliferate (Nair *et al.*, 2006).

CONCLUSION

The biological parameters such as individual adult worm weight, length of individual worm, number of cocoons, juveniles and adult worms and worm biomass have significantly increased. Temperature has significantly decreased. pH has come to neutral. The Nitrogen (N), Phosphorus (P), Potassium (K) has significantly increased. The microbial content of *E. coli, Staphylococcus aureus, Klebsiella pneumoniae, Bacillus cereus, and Bacillus subtilis* has significantly decreased making the vermicompost a rich manure.

Biomedical waste Management is often ignored due to the lack of knowledge on the hazards it poses to the environment and people inhabiting it. As a result of this, healthcare institutions do not take utmost care in disposing the biomedical waste properly mainly due to the prohibitive cost involved in it. The proper management of biomedical waste is a concern that has been recognized by both government agencies and the Nongovernment organizations. Vermiculture is a substantial way of reducing wastes, producing fertilizers and maintaining the balance of the ecological environment. Vermicomposting can produce high-quality fertilizers which are better compared to other commercial fertilizers in the market.

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