

ECOFRIENDLY TREATMENT OF BIOMEDICAL WASTE USING *EUDRILUS EUGENIAE*Greeshma G.², Selvalakshmi M.² and Pawlin Vasanthi Joseph^{1*}¹Associate Professor and Head, Department of Zoology, Nirmala College for Women (Autonomous), Coimbatore-641018. Tamilnadu, India.²Post Graduate Student, Department of Zoology, Nirmala College for Women (Autonomous), Coimbatore-641018 Tamilnadu, India.***Corresponding Author: Pawlin Vasanthi Joseph**

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ABSTRACT

An average of 40 tonnes of biomedical waste from 8,000 private hospitals and 2,200 government hospitals in the Kerala State is brought to Malampuzha on a daily basis. The enormous quantity of solid waste generation with its ever increasing trend is one of the growing problems of concern in both developed and developing countries. Vermicomposting is a simple biotechnological process of composting, in which certain species of earthworms are used to enhance the process of waste conversion and produce a better end product. The aim of the present work was to study the vermicomposting of biomedical waste from hospitals by employing *Eudrilus eugeniae*. The biological, physical and nutrient parameters of the vermicompost was analysed and the depletion rate of pathogenic bacteria in the vermicompost was assessed. All the biological parameters such as individual adult worm weight, length, total number of adult worms, number of cocoons, juveniles and total worm biomass increased. Temperature significantly decreased in the experiment. pH has come to neutral in the experiment. Moisture content has significantly increased in the samples. Nutrient parameters such as nitrogen, phosphorus and potassium have also significantly increased. All five pathogens have reduced significantly. Low capital investment and relatively simple technologies make vermicomposting practical for less developed agricultural regions.

KEYWORDS: Vermicomposting, Bio-medical waste, cocoons, juveniles, total worm biomass, nutrient parameters, biological parameters.

INTRODUCTION

India produces about 3000 million tons of wastes annually and nearly 60% of this constitutes decomposable organic waste. An average of 40 tonnes of biomedical waste from 8,000 private hospitals and 2,200 government hospitals in the Kerala State is brought to Malampuzha on a daily basis. On an average, about 39 tonnes of biomedical waste reaches the 'Image' disposal plant. The enormous quantity of solid waste generation with its ever increasing trend is one of the growing problems of concern in both developed and developing countries. In any environment, recycling of the degradable waste is the natural way of replenishing it with the friendly ingredients from wastes (Amita and Joseph, 2017).

Bio-medical waste means any waste generated during diagnosis, treatment or immunization of human beings or animals. Inappropriate handling of biomedical waste may have serious public health consequences and a significant impact on the environment. Major hospitals contribute substantially to the quantum of generated biomedical waste. Smaller hospitals, nursing homes, clinics,

pathological laboratories and blood banks also have major contribution to biomedical waste. Wastes targeted for precautions during handling and disposal include sharps (needles or scalpel blades), pathological wastes (anatomical body parts, microbiology cultures and blood samples) and infectious wastes (items contaminated with body fluids and discharges such as dressing, catheters and I.V. lines) (Askarain *et al.*, 2004; Remy, 2001).

Vermicomposting is a simple biotechnological process of composting, in which certain species of earthworms are used to enhance the process of waste conversion and produce a better end product. Vermicomposting is a process of production of compost by breeding earthworms, resulting in homogeneous and stabilized humus used as manure. The earthworm is very beneficial for the soil fertility and for maintaining the earthworm in the soil, soil moisture and organic matter should be at optimum temperature level. In short, earthworms, through a type of biological alchemy, are capable of transforming garbage into 'gold' (Tara Crescent, 2003).

From such a point of view the analysis of nutrient contents in biomedical waste treated with earthworm for plant growth is very important. Vermicompost, due to the aggregate nature of the worm castings has appreciable water holding capacity and its use leads to improved soil structure. Vermicompost itself is highly valued by gardeners all over the world and has a significant market value.

Mani *et al.*, (2001), found that bio-medical waste (BMW) is any solid or liquid waste, which may present a threat of infection to humans. It is generated from healthcare establishments such as hospitals, blood banks, laboratories and research institutes, veterinary hospitals. Hospital waste is highly infectious and can be a serious threat to human health if not managed in a scientific and discriminate manner. It has been roughly estimated that of the 10 kg of waste generated in a hospital at least 2 kg would be infected (Mani *et al.*, 2001). Hospital is one of the most complex institutions which are frequently visited by people from every walk of life in the society (Dilip *et al.*, 2013). The aim of the present work was to study the vermicomposting of biomedical waste from hospitals by employing *Eudrilus eugeniae*. The biological, physical and nutrient parameters of the vermicompost was analysed and the depletion rate of pathogenic bacteria in the vermicompost was assessed.

MATERIALS AND METHODS

Experimental Design

Biomedical waste was collected from hospitals in Palakkad, Kerala and treated with 5% NaOCl. This included blood stained cotton pieces, pus and body fluids, antiseptics/ antibiotics used for dressing of wounds, spilled liquid and tissues. The initial decomposition was done by mixing it with cow dung slurry in mud pots. Ten worms were introduced into each mudpot. Natural composting (without worms) was considered as control. Vermicomposting was carried out for 60 days. The experiment was carried out in triplicates. Analysis was carried out after every 15 days.

METHODOLOGY

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

Individual adult worm weight, Individual length, Total number of adult worms, Number of cocoons, Number of juveniles, Total worm biomass

Physical Parameters

pH – Digital pH meter (Standard method), 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 (Pelcazaret *et al.*, 1986; Swanson *et al.*, 1992): Study of 5 pathogens - *Escherichia coli*, *Staphylococcus aureus*, *Klebsiella pneumonia*, *Bacillus Cereus*, *Bacillus Subtilus*

RESULTS AND DISCUSSION

Biological Parameters

Weight of individual adult worm for 15 days is 0.80 ± 0.10 and on the 60th day it has increased to 1.50 ± 0.10 . Individual length of adult worm for 15 days is 8.57 ± 0.5 , 30 days (9.13 ± 0.21), 45 days (9.27 ± 0.15) and for 60 days it has increased to 10.17 ± 0.15 . Total number of earthworms for 15 days is 3.00 ± 1.00 , and it has increased to 31.33 ± 3.06 for the 60th day. Number of cocoons was 17.67 ± 1.33 for 15 days and has increased to 62.33 ± 2.08 for the 60th day. Number of juveniles also increased up to 71.33 ± 2.08 . Its initial number is 22.67 ± 2.08 . Total worm biomass for 15 days is 3.10 ± 0.10 and has increased to 4.37 ± 0.15 on the 60th day.

Physico-Chemical Parameters

Temperature: The temperature of the control for 15 days is 33.0 ± 1.0 , for 30 days it is 32.03 ± 1.00 . On the 45th day it has decreased to 30.76 ± 1.68 and on the 60th day it is 27.30 ± 0.95 ($P < 0.01$). The temperature of the sample for 15 days is 31.66 ± 1.52 and on the 60th day it has decreased to 25.03 ± 1.00 ($P < 0.01$). The one way ANOVA for temperature is significant at 1% level.

pH: The pH of the control on the 15th day is 5.90 ± 0.10 , 60th day it has increased to 6.36 ± 0.15 ($P < 0.01$). An acidic pH is observed. The experimental value for 15 days is 6.13 ± 0.15 , 30th day is 6.66 ± 0.15 , it has increased to 6.80 ± 0.10 on the 45th day. An acidic pH is observed on the 45th day and on the 60th day it is neutral, 7.10 ± 0.10 ($P < 0.01$). The one way ANOVA for pH is significant at 1% level.

Moisture content: The moisture content of the control for 15 days is 61.06 ± 0.90 , for 30 days it is 62.66 ± 0.41 , 45th day it has increased to 63.60 ± 0.45 , and 60th day it is 64.16 ± 0.37 ($P < 0.05$). The moisture content of the sample for 15 days is 60.10 ± 0.10 , and on the 60th day it has increased to 65.76 ± 0.68 ($P < 0.05$). The one way ANOVA for moisture content is significant at 5% level.

NUTRIENT PARAMETERS

Nitrogen: The nitrogen content of the control for 30 days is 0.41 ± 0.01 , 60th day 0.4 ± -0.01 ($P < 0.01$). The experimental value on the 30th day has increased to 0.71 ± 0.01 , and on the 60th day 0.082 ± 0.02 ($P < 0.01$). The one way ANOVA for nitrogen is significant at 1% level.

Phosphorus: The phosphorus content of the control is 0.03 ± 0.01 on the 30th day, and 0.07 ± 0.01 ($P < 0.01$) on the 60th day. The experimental value on the 30th day is 0.26 ± 0.01 , and has increased to 0.34 ± 0.02 ($P < 0.01$) on the 60th day. The one way ANOVA for phosphorus is significant at 1% level.

Potassium: The potassium content of the control on the 30th day is 0.09 ± 0.01 , and it is 0.12 ± 0.02 ($P < 0.01$) on the 60th day. The potassium content of the experimental sample is 0.25 ± 0.02 on the 30th day, and has on the 60th day increased to 0.26 ± 0.02 ($P < 0.01$). The one way ANOVA for potassium is significant at 1% level.

MICROBIAL PARAMETERS

Vermicomposting, reduced the pathogen levels more than the normal composting.

Escherichia coli: The *E.coli* count of the control on the 1st day is 8.06 ± 1.00 , on the 30th day 7.20 ± 1.05 , and is 6.10 ± 0.90 ($P < 0.05$) on the 60th day. In the experimental sample *E.coli* count is 8.10 ± 0.95 on the 1st day, on the 30th day the count is 6.26 ± 0.64 , and on the 60th day it has decreased to 2.36 ± 1.58 ($P < 0.05$).

Staphylococcus aureus: The *S.aureus* count of the control is 6.16 ± 1.15 on the 1st day, 5.10 ± 0.95 on the 30th day, and on the 60th day 5.06 ± 2.10 ($P < 0.05$). The experimental value on the 1st day is 9.06 ± 2.05 , 30th day is 5.46 ± 0.25 , and the 60th day it has decreased to 2.66 ± 1.52 ($P < 0.05$).

Klebsiella pneumoniae: The *K. pneumoniae* count of the control on the 1st day is 6.40 ± 1.44 , and on the 60th day it is 3.76 ± 2.05 ($P < 0.01$). In the sample its count is 5.46

± 1.51 on the 1st day, for the 30th day it is 3.90 ± 2.66 , and on the 60th day it has decreased to 2.10 ± 0.90 ($P < 0.01$).

Bacillus Cereus: The count of *B.cereus* of the control for the 1st day 6.36 ± 1.00 , for 30 days it is 5.33 ± 0.61 , and for 60 days it is 5.23 ± 1.85 ($P < 0.01$). The experimental count for 1st day 3.06 ± 1.90 , on the 30th day it has decreased to 2.40 ± 1.53 , and on the 60th day it has also decreased to 1.33 ± 1.52 ($P < 0.01$).

Bacillus Subtilis: The *B.subtilis* count of the control is 3.80 ± 1.67 for the 1st day, on the 30th day is 3.43 ± 1.42 , and on the 60th day it has decreased to 2.83 ± 2.28 ($P < 0.01$). The count of *B.subtilis* of the experimental sample on the 1st day is 4.03 ± 1.05 , it is 4.03 ± 2.05 for 30days, and on the 60th day it has decreased to 2.36 ± 1.51 ($P < 0.01$).

The one way ANOVA for the bacterial count is significant at 5% and 1% level.

Table. 1: Growth parameters of earthworm in Biomedical waste.

Parameters	Days of Treatment			
	15	30	45	60
Earthworm-weight (g)	0.80 ± 0.10	1.03 ± 0.15	1.30 ± 0.10	1.50 ± 0.10
Earthworm-height (cm)	8.57 ± 0.51	9.13 ± 0.21	9.27 ± 0.15	10.17 ± 0.15
Total no.of earthworm	3.00 ± 1.00	5.00 ± 1.00	12.67 ± 1.53	31.33 ± 3.06
No.of cocoons	17.67 ± 1.53	31.67 ± 1.53	47.00 ± 1.00	62.33 ± 2.08
No.of juveniles	22.67 ± 2.08	38.33 ± 1.53	46.00 ± 2.00	71.33 ± 2.08
Total worm biomass (g)	3.10 ± 0.10	4.00 ± 0.10	4.23 ± 0.21	4.37 ± 0.15

Values are Mean \pm Standard Deviation

Table. 2: Temperature of vermicompost during experimental period.

Samples	Days of Treatment			
	15	30	45	60
Control	$33.0 \pm 1.0^*$	$32.03 \pm 1.00^{**}$	$30.76 \pm 1.68^*$	$27.30 \pm 0.95^*$
Experiment	$31.66 \pm 1.52^*$	$29.20 \pm 0.20^{**}$	$27.96 \pm 0.15^*$	$25.03 \pm 1.00^*$

Values are Mean \pm Standard Deviation * - Significant at $P < 0.05$ level; ** - Significant at $P < 0.01$ level.

Table. 3: pH of vermicompost during experimental period.

SAMPLES	DAYS OF TREATMENT			
	15	30	45	60
Control	$5.90 \pm 0.10^*$	$6.26 \pm 0.15^{**}$	$6.26 \pm 0.15^{**}$	$6.36 \pm 0.15^{**}$
Experiment	$6.13 \pm 0.15^*$	$6.66 \pm 0.15^{**}$	$6.80 \pm 0.10^{**}$	$7.10 \pm 0.10^{**}$

Values are Mean \pm Standard Deviation * - 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	61.06 ± 0.90 NS	62.66 ± 0.41 NS	63.60 ± 0.45 NS	$64.16 \pm 0.37^*$
Experiment	60.10 ± 0.10 NS	62.70 ± 0.65 NS	64.06 ± 0.25 NS	$65.76 \pm 0.68^*$

Values are Mean \pm Standard Deviation; * - Significant at $P < 0.05$ level; NS – Not Significant.

Table. 5: Nutrient parameters of vermicompost during experimental period.

Parameters	Days of Treatment		
	Sample	30	60
Nitrogen	Control	0.41 \pm 0.01NS	0.44 \pm 0.01NS
	Experiment	0.71 \pm 0.01NS	0.82 \pm 0.02NS
Phosphorus	Control	0.03 \pm 0.01NS	0.07 \pm 0.01NS
	Experiment	0.26 \pm 0.01NS	0.34 \pm 0.02NS
Potassium	Control	0.09 \pm 0.01**	0.12 \pm 0.02**
	Experiment	0.25 \pm 0.02**	0.26 \pm 0.02**

Values are Mean \pm Standard Deviation; ** - Significant at $P < 0.01$ level; NS – Not Significant.

Table. 6: Microbial content of vermicompost during experimental period.

Pathogens	Samples	Days of Treatment		
		1	30	60
Escherichia coli	Control	8.06 \pm 1.00NS	7.20 \pm 1.05NS	6.10 \pm 0.90*
	Experiment	8.10 \pm 0.95NS	60.26 \pm 0.64NS	2.36 \pm 1.58*
Staphylococcus aureus	Control	6.16 \pm 1.15*	5.10 \pm 0.95NS	5.06 \pm 2.10NS
	Experiment	9.06 \pm 2.05*	5.46 \pm 0.25NS	2.66 \pm 1.52NS
Klebsiella pneumonia	Control	6.40 \pm 1.44NS	4.40 \pm 1.57NS	3.76 \pm 2.05NS
	Experiment	5.46 \pm 1.51NS	3.90 \pm 2.66NS	2.10 \pm 0.90NS
Bacillus cereus	Control	6.36 \pm 1.00*	5.33 \pm 0.61*	5.23 \pm 1.85*
	Experiment	3.06 \pm 1.90*	2.40 \pm 1.53*	1.33 \pm 1.52*
Bacillus subtilis	Control	3.80 \pm 1.67*	3.43 \pm 1.42*	2.83 \pm 2.28*
	Experiment	4.03 \pm 1.05*	4.03 \pm 2.05*	2.36 \pm 1.51*

Values are Mean \pm Standard Deviation; * - Significant at $P < 0.05$ level; NS – Not Significant.

Table. 7: One way ANOVA for physico-chemical parameters analyzed during the Experimental period.

Parameters	Days	Df	SS	MS	F	P	CV%
Temperature	15	3	2.666667	2.666667	64.0000	0.015*	0.63
	30		12.041667	12.041667	37.4352	0.026*	1.85
	45		11.760000	11.760000	24.2474	0.039*	2.37
	60		7.706667	7.706667	58.5316	0.017*	1.39
pH	15	3	0.081667	0.81667	49.0000	0.020*	0.68
	30		0.601667	0.601667	361.0000	0.000**	0.64
	45		0.426667	0.426667	256.0000	0.000**	0.62
	60		0.806667	0.806667	484.0000	0.000**	0.61
Moisture content	15	3	1.401667	1.401667	30.0357	0.032*	0.36
	30		0.400417	0.400417	25.9730	0.036*	0.20
	45		0.326667	0.326667	28.0000	0.034*	0.17
	60		3.840000	3.840000	36.5714	0.026*	0.50

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. 8: One way ANOVA for nutrient parameters during the experimental Period.

Parameters	Days	Df	SS	MS	F	P	CV%
Nitrogen	30	5	0.135000	0.135000	3039.9297	0.000**	0.00
	60		0.220417	0.220417	3306.2500	0.000**	1.29
Phosphorus	30	5	0.077067	0.077067	4624.0000	0.000**	2.78
	60		0.109350	0.109350	2187.0000	0.000**	3.39
Potassium	30	5	0.038400	0.038400	768.0000	0.000**	4.16
	60		0.028017	0.028017	1681.0000	0.000**	2.09

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.

Table. 9: One way ANOVA for microbial parameters during experimental period.

Pathogens	Days	df	SS	MS	F	P	CV%
<i>Escherichia coli</i>	1	8	0.166667	0.166667	25.0000	0.038*	0.99
	30		1.306667	1.306667	112.0000	0.000**	1.60
	60		20.906667	20.906667	18.9773	0.049*	24.79
<i>Staphylococcus aureus</i>	1	8	12.615000	12.615000	93.4444	0.011**	4.82
	30		0.426667	0.426667	19.6923	0.047*	2.74
	60		8.640000	8.640000	36.0000	0.027*	12.67
<i>Klebsiella pneumoniae</i>	1	8	1.306667	1.306667	112.0000	0.000**	1.82
	30		0.375000	0.375000	75.0000	0.013**	1.70
	60		4.166667	4.166667	131.5789	0.000**	6.07
<i>Bacillus cereus</i>	1	8	16.335000	16.335000	38.8929	0.025*	13.74
	30		12.906667	12.906667	29.8996	0.032*	16.99
	60		22.815000	22.815000	240.1579	0.000**	9.39
<i>Bacillus subtilis</i>	1	8	0.081667	0.81667	49.0000	0.020*	1.04
	30		0.666667	0.666667	100.0000	0.010**	2.17
	60		0.326667	0.326667	196.0000	0.000**	1.57

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: There was an increase in the growth of earthworms. The earthworms' introduction in to the waste ingests the same as feed and assimilates a part of the same for their growth and reproduction. Compared to the first cycle the following cycle showed an increase in all the parameters. This shows that prior adaptation to any given material is essential for them to accept as feed and to continue their life cycle. The mean individual length and live weight, mean growth rate of an individual (mg/day), individual and total biomass gain, reproduction rate (cocoon worm⁻¹ day⁻¹), fecundity rate (worm cocoon⁻¹ day⁻¹), total cocoon, juveniles and adult numbers have increased.

The newspaper vermicompost had high levels of earthworm numbers, earthworm biomass and cocoon production while growth rate of worms was found to be higher in written paper vermicompost. (Amita and Joseph., 2017). The increase in body weight of earthworm species during vermicomposting process could be due to the substrate quality or could be related to fluctuating environmental conditions (Reinecke *et al.*, 1992; Edwards *et al.*, 1998; Suthar, 2007).

The indigenous species, *Perionyx excavates*, exhibited better growth and reproduction performance compared to the other two exotic species (Garget *et al.*, 2006). The higher numbers of cocoons, juveniles, and adults collected from the vermicompost processed by *Perionyx excavatus*, were probably because its indigenous nature being acclimatized to the abiotic environmental conditions extremely well when compared to other species. The growth rate difference between the species was probably due to the species-specific growth patterns or could be related to the feed quality and preferences by individual species of earthworm (Suthar and Singh, 2008).

Temperature: At the start of the experiment, the temperature of the substrate was high and then decreased gradually as the composting process progressed. The heat released by the oxidative action of intensive microbial activity on the organic matter resulted in the rise in temperature during the first mesophilic phase of composting process (Peigne and Girardin, 2004).

The temperature of the following thermophilic phase rose up above 40°C reaching about 60°C when most of the organic matter was degraded with the help of thermophilic bacteria and fungi, consequently depleting most of the oxygen.

The thermophilic phase was followed by cooling phase, when compost maturation stage occurred and compost temperature dropped to that of the ambient (Zibilske, 1999). Then, the decreasing trend of temperature with the progress of composting process occurred, which was probably due to the decreased bacterial activity. It may also be attributable to regular sprinkling of water.

pH

The near-neutral pH of vermicompost may be attributed to the secretion of NH₄⁺ ions that reduce the pool of H⁺ ions (Haimi and Huhta, 1987) and the activity of calciferous glands in earthworms containing carbonic anhydrase that catalyzes the fixation of CO₂ as CaCO₃, thereby preventing the fall in pH (Kale *et al.*, 1982).

The increased trend of pH in the vermicompost and compost samples is in consistence with the findings of Tripathi and Bhardwaj (2004) and Loh *et al.*, (2005), which was due to higher mineralization. The increased pH during the process was probably due to the degradation of short-chained fatty acids and ammonification of organic N (Guoxue *et al.*, 2001 and Tognetti *et al.*, 2005).

High solubility of nutrients in earthworm casts increase the pH of cast (Barley and Jennings, 1959). This could be another reason for the rise in pH of the substrates observed in the present study. A neutral pH is suggested for efficient degradation and for biological augmentation of earthworms (Amita and Joseph, 2017). The observed neutral pH in the present study is in par with the above results, which was found to be favourable for the increasing biomass.

Moisture Content

Edwards and Bater (1992) reported that optimum moisture content for growth of earthworms - *Eisenia fetida*, *Eudrilus eugeniae* and *Perionyx excavates* - was 85% in organic waste management. The rate of mineralization and decomposition becomes faster with the optimum moisture content (Singh *et al.*, 2004).

According to Liang *et al.*, 2003, the moisture content of 60–70% was proved to having maximal microbial activity, while 50% moisture content was the minimal requirement for rapid rise in microbial activity. Vermicompost samples during the present study showed higher moisture content than the compost and substrate, which may be due to their high absorption capacity, and may also be because of assimilation rate by microbial population indicating the higher rate of degradation of waste by earthworms (Pattnaik and Reddy, 2009).

Nitrogen

The nutrient level of vermicompost depends on the nature of the organic waste used as the food source for earthworms (Kawaguchi and Nishi, 2007). Nitrogen is an essential component of all protein. Hand *et al.*, (1988), has reported that nitrogen mineralization was greater in the presence of earthworms, and this mineral nitrogen is retained in nitrate form.

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 uric acid as in a fluid excreted from the nephridiopores (Edwards and Lofty, 1977).

Graff (1971), reported that the excreta of earthworms had more nitrogen considerably in casts than the soil. Curry *et al.*, (1995) observed that the earthworms would contribute an addition of 3.4–4.1g of mineral nitrogen to the soil through excretion, mucus production and soil ingestion. Bhatnagar and Patla (1996) revealed that about 6% more nitrogen was made available to plants by worm excreta.

Phosphorus

The initial and final phosphorus content in the compost show that composting has increased phosphorus concentration over control. Phosphorus was found to be more in casts than the surrounding soil (Graff, 1971). Bhatnagar and Patla (1996) reported that 15–30% more phosphorus was made available to plants by worm

activity. Vermicompost obtained individually from cow dung, sugarcane trash, pig manure and horse manure had higher levels of phosphorus compared to control (Jambhekar, 1992).

Amita and Joseph, 2016 reported that the final vermicompost of paper waste treatment was rich in phosphorus. The total phosphorus concentration in the product was increased by earthworm activity when compared to its level in the control. The raising level of available phosphorus indicates the mineralization of phosphorus organic compounds during the process of vermicomposting (Hartenstein, 1981; Mitchell, 1997). The total phosphorus was higher in the vermicompost harvested at the end of the experiment compared to that of the initial substrate (Kaushik and Garg, 2003; Suthar, 2007; Manna *et al.*, 2003).

Potassium

The initial and final potassium in different concentrations of biomedical waste revealed that potassium increased progressively more than the control. Increase of potassium was reported by Swati and Reddy (2010) during vermicomposting of urban green wastes. Extractable potassium was found highest in rice straw vermicompost. The increase of the extractable potassium may be attributed to both the actions of earthworms and microbes activities during vermicomposting.

Vermicomposting proved to be an efficient process for recovering higher K from organic waste. The increase in K of the vermicompost in relation to that of the simple compost and substrate 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, which may have caused its increase (Rao *et al.*, 1996). The microorganisms present in the worm's gut probably converted insoluble K into the soluble form by producing microbial enzymes (Kaviraj and Sharma, 2003).

Microbial Study

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. 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 *et al.*, (2010), investigated the reduction of pathogens' numbers in tank's septic sludge in vermicomposting and showed that the pathogens numbers have decreased considerably which is in the same line with results of the present study. Nair *et al.*, (2007), studied that vermicomposting leads to greater

reduction of pathogens after three months upon storage. Amita and Joseph, (2017), observed a significant reduction of coliforms as the substrate enters the food chain of the earthworm.

Warm temperature also promotes chemical and microbial activity in the substrate, and the increased microbial activity tends to consume the available oxygen, with the negative effects on the survival of earthworms (Dominguez and Edwards, 2011). 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-composting process did not reach a high enough temperature, it was possible not only that pathogens may be sufficiently inactivated, but also that they would even proliferate (Nair *et al.*, 2006).

CONCLUSION

All the biological parameters such as individual adult worm weight, length, total number of adult worms, number of cocoons, juveniles and total worm biomass increased. Temperature has significantly decreased in the experiment. pH has come to neutral in the experiment. Moisture content has significantly increased in the samples. Nutrient parameters such as nitrogen, phosphorus and potassium have significantly increased. All five pathogens have reduced significantly.

Vermicompost is superior to traditional compost for its ability to improve soil structure and increase its water-holding capacity. Vermicompost improves soil aeration, and root growth. It enhances germination, plant growth and crop yield. Bio waste conversion reduces waste flow to landfills. Low capital investment and relatively simple technologies make vermicomposting practical for less developed agricultural regions. It helps to close the 'metabolic gap' through recycling waste on-site. Production reduces greenhouse gas emissions such as methane and nitric oxide.

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REFERENCES

1. Amita Paul C and Joseph P V. Study of biological parameters of paper waste degraded through vermicomposting in an institutional setup. *Eur J Pharm Med Res*, 2017; 4(6): 593-602.
2. Amita Paul C and Joseph P V. NPK ratio of paper waste degraded through vermicomposting in an institutional setup. *Proceedings of the National seminar on Eco waste management and Nanobiology*, 2016; pgs 1-4, ISBN 978-93-86176-36-3.
3. Askarian M, Vakili M, Kabir G. Hospital waste management status in university hospitals of the Fars province, Iran. *Int.J. Environ. Health Res.*, 2004; 14: 295-305.
4. Remy L. Managing Hospital Waste is a Big. Nasty Deal, *Great Western Pacific Costal Post*, 2001.
5. Tara Crescent. Vermicomposting. *Development Alternatives (DA) Sustainable Livelihoods*, 2003; (<http://www.dainet.org/livelihoods/default.htm>).
6. Mani S K, Bansal A K, Banerjee R and Choudhry A. Scenario of Biomedical Waste Management in Delhi - a report by the Centre for Environment Education to Hazardous Waste Management Division of the Ministry of Environment and Forests. GOI, New Delhi, India, 2001.
7. Dilip Kumar Aske. Biomedical waste management through Vermitechnology. *Environment Conservation Journal*, 2013; 14(3): 105-107.
8. Dinesh M S, Geetha K S, Vaishnavi V, Radha D, Kale and Krishna Murthy V. Ecofriendly treatment of biomedical wastes using epigeic earthworms. *Journal of ISHWM*, 2010; 9(1): 5-20.
9. Karl Fischer. 'Neues Verfahren zur massanalytischen Bestimmung des Wassergehaltes von Fluessigkeiten und festen koerpern'. *Angew. Chemie*, 1935; 48-394.
10. Kjeldahl J. New method for the determination of nitrogen in organic compounds, *Z. anal. Chem.*, 1883; 22(1): 366-383.
11. Bray R H and Kurtz L T. Determination of total, organic, and available forms of phosphorus in soils, *Soil Science*, 1945; 59: 39-45.
12. Engelbrecht RM and McCoy FA. "Determination of potassium by Tetraphenylborate Method". *Anal. Chem*, 1772; 28 (11).
13. Pelczar MJ, Chan EC S. Jr. and Kreig NR. *Microbiology*. Int. Edn.: McGraw-Hill, 1986.
14. Swanson K M J, Busta F F, Peterson E H and Johnson M G. Colony count methods. In: Vanderzant C and Splittstoesser DF, (Eds.), *Compendium of Methods for the Microbiological Examination of Foods*. 3rd ed. USA: American Public Health Association, 1992; 75-95.
15. Suthar S and Singh S. Vermicomposting of domestic waste by using two epigeic earthworms (*Perionyx excavatus* and *Perionyx sansibaricus*) *IntJ Evniron Sci and Technol*, 2008; 5: 99-106.
16. Reinecke A J, Viljoen S A and Saayman R J. The suitability of *Eudrilus eugeniae*, *Perionyx excavatus* and *Eisenia foetida* (Oligochaeta) for vermicomposting in Southern Africa in terms of their temperature requirements. *Soil. Biol. Biochem.*, 1992; 24: 1295-1307.
17. Edwards C A, Dominguez J and Neuhauser E F. Growth and reproduction of *Perionyx excavatus* (Megascolecidae) as factors in organic waste management. *Biol Fertil Soils*. 1998; 27: 155-161.
18. Suthar S. Influence of different food sources on growth and reproduction performance of composting epigeics: *Eudrilus eugeniae*, *Perionyx excavatus* and

- Perionyx sansibaricus. Appl. Ecol. Environ. Res, 2007; 579-92.
20. Suthar S. Nutrients changes and biodynamic of epigeic earthworms Perionyx excavatus during recycling of some agricultural wastes. Bioresource Technology, 2007; 98 (8): 1608-1614.
 21. Garg VK, Yadav YK, Sheoran A, Chand S and Kaushik P. Livestock excreta management through vermicomposting using an epigeic earthworm Eisenia foetida. Environmentalist, 2006; 26: 269-276.
 22. Peigne J, Girardin P. Environmental impacts of farm-scale composting practices. Water Air Soil Pollut, 2004; 153: 45-68.
 23. Zibilske LM. Composting of organic wastes. In: Sylvia DM, Fuhrmann JJ, Hartel PG, Zuberer DA (Ed.) Principles and Applications of soil microbiology. Prentice Hall Publication, 1999; 482-497.
 24. Haimi J and Huhta V. Comparison of composts produced from identical wastes by vermistabilization and conventional composting. Pedobiologia, 1987; 30 (2): 137-144.
 25. Kale R D, Bano K, Krishnamoorthy R V. Potential of Perionyx excavatus for utilizing organic wastes. Pedobiologia, 1982; 23: 419-425.
 26. Tripathi G and Bhardwaj P. Comparative studies on biomass production, life cycles and composting efficiency of Eisenia foetida (Savigny) and Lampitomaauritii (Kinberg). Bioresource Technology, 2004; 92: 275-278.
 27. Loh T C, Lee Y C, Liang J B, and Tan D, "Vermicomposting of cattle and goat manures by Eisenia foetida and their growth and reproduction performance," Bioresource Technology, 2005; 96(1): 111-114.
 28. Guoxue L, Zhang F, Sun Y, Wong J W C and Fang M. Chemical evaluation of sewage composting as mature indicator for composting process, Water air soil sludge pollution, 2001; 132: 333-345.
 29. Tognetti C, Laos F, Mazarrino MJ and Hernandez MT. Composting vs. vermicomposting: A comparison of end product quality. Compost Science and utilization, 2005; 3: 6-13.
 30. Barley K P and Jennings A. Earthworms and Soil Fertility III. The Influence of Earthworms on the Availability of Nitrogen. Aust J. Agr. Res, 1959; 10: 364-370.
 31. Bhatnagar R K and Palta R K. Earthworm-vermiculture and Vermicomposting. New Delhi, Kalyani Publishers, 1996.
 32. Edwards C A and Bate J E. "The use of earthworms in environmental management," Soil Biology and Biochemistry, 1992; 24(12): 1683-1689.
 33. Singh N B, Khare A K, Bhargava D S, and Bhattacharya S. "Optimum moisture requirement during vermicomposting using Perionyx excavatus," Applied Ecology and Environmental Research, 2004; 2: 53-62.
 34. Pattnaik S and Reddy VM. Nutrient Status of Vermicompost of Urban Green Waste and Floral Waste Processed by three species of Earthworms namely, Eudrilus eugeniae, Eisenia foetida and Perionyx excavatus. Appl. Environ. Soil Sci., 2009; 1-13.
 35. Kawaguchi S and Nishi S. Nutritional and Microbial parameters of Earthworm cast, Termite Mound and Surrounding Bulk. Soil. J. Fac. Agr., Kyushu Uni, 2007; 52 (2): 367-369.
 36. Hand P, Hayes WA, Satchell J E, Frankland J C, Edward C A and Neuhauser E F. The vermicomposting of cow slurry. Earthworms in waste and environmental management, 1988; 31: 49-63.
 37. Curry J P, Byrne D and Boyle K E. The earthworm population in winter cereal field and its effects on soil and nitrogen turnover. Biol. Fertil. Soils., 1995; 19: 166-172.
 38. Edwards C A and Lofty J R. Biology of earthworms. 1sted. Chapman and Hall, London, 1977; 333.
 39. Graff O. Stickstoff, Phosphor und Kalium in der Regenwurmlosung auf der Wiesenversuchfläche des Sollingprojektes. In IV Colloquium Pedobiologie, D'Aguilar, J. (Ed.), Paris: Institut National des Recherches Agricultrices, 1971; 503-511.
 40. Jambhekar H. Use of earthworm as a potential source to decompose organic wastes. Proc. Nat. Sem. Org.Fmg., Coimbatore, India, 1992; 52-53.
 41. Hartenstein R. Soil macroinvertebrates, aldehyde oxidase, catalase, cellulose and peroxidase. Soil Biology and Biochemistry, 1981; 15: 51-54.
 42. Mitchell A. Production of Eisenia fetida and vermicompost from feed-lot cattle manure. Soil Biol Biochem, 1997; 29: 763-766.
 43. Kaushik P and VK Garg. Vermicomposting of mixed solid textile mill sludge and cow dung with the epigeic earthworm Eisenia foetida. Biores. Tech., 2003; 90(3): 311-316.
 44. Manna M C, Jha S, Ghosh P K and Acharya C L. Comparative efficacy of three epigeic earthworms under different deciduous forest litters decomposition. Bioresource Technology, 2003; 88: 197-206.
 45. Swati P and Reddy M V. Nutrient status of vermicompost of urban green waste processed by three different earthworm species- Eisenia foetida, Eudrilus eugeniae and Perionyx excavatus. Appl. Environ. Soil Sci., 2010; 1-13.
 46. Rao S, Rao A S and Takkar P N. Changes in different forms of K under earthworm activity In. Proceedings of the National Seminar on Organic Farming and Sustainable Agriculture. Ghaziabad, India, 1997; 9-11.
 47. Kaviraj and Sharma S. Municipal solid waste management through vermicomposting employing exotic and local species of earthworms. Bioresource Technology, 2003; 90: 169-173.
 48. Rodriguez-Canchè LG, Vigueros C, Maldonado-Montiel T and Martinez-Sanmiguel M. Pathogen

- reduction in septic tank sludge through vermicomposting using *Eisenia foetida*. *Bioresource Technology*, 2010; 101: 3548-3553.
49. Nair J, Mathew K and Ho G. Earthworms and composting worms – Basics towards composting applications. Paper at Water for All Life – A Decentralised Infrastructure for a Sustainable Future., Marriott Waterfront Hotel, Baltimore, USA, 2007; 12-14.
 50. Dominguez J and Edwards CA. Biology and ecology of earthworm species used for vermicomposting. In C. A. Edwards, N. Q. Arancon, & R. Sherman (Eds.), *Vermiculture Technology Florida*, CRC Press Taylor and Francis Group, 2011; 249-261.
 51. Nair J, Sekiozoic V and Anda M. Effect of pre-composting on vermicomposting of kitchen waste. *Bioresource Technology*, 2006; 97(16): 2091-2095.