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## Bioremediation of temple waste (nirmalya) by vermicomposting in a metropolitan city like Mumbai

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### Abstract

In India, million tons of temple waste (nirmalya) is produced every day. The waste collected from temple mainly consists of flowers, leaves, fruits, honey, coconuts, camphor, jaggery, milk etc. which is released in the water bodies or dumped at the available land spaces, thereby leading to severe environmental pollution and health hazards. Bioremediation of nirmalya can be carried out by vermicomposting. Vermicomposting is an eco-friendly process of efficiently converting organic waste into compost with the help of soil microorganism and earthworms. In our work Nirmalya was taken from a temple in South Mumbai, which was pre-composted at 30°C and used as a substrate for vermicomposting by earthworm species *Eisenia foetida* for 90 days. The chemical analysis of the vermicompost showed its pH (7.2), the organic carbon content (8.57%), N (0.49%), total P (0.5%), K (0.16%), C: N ratio (17.489) and also contained sufficient concentration of microelements like zinc, manganese, iron and copper. The total bacterial count of vermiwash was found to be  $3 \times 10^9$  cfu/ml. The bacteria which were isolated from vermiwash showed various enzyme activities like protease, cellulase, phosphatase, amylase, gelatinase and lipase. The presence of nitrogen fixing bacteria like *Azotobacter* and *Rhizobium* from vermiwash was also demonstrated. The vermicompost obtained was checked for its effect on the growth of the test plants like *Tagetes erecta* and *Solanum melongena* using pot culture studies. One of the bacterial isolates was identified as *Bacillus amyloliquefaciens* HY10 by morphological, cultural, biochemical and 16s rRNA sequence analysis which showed protease (32.53units/ml) and lipase activity (3.177units/ml).

**Keywords:** Vermicompost; *Eisenia foetida*; Nirmalya; *Bacillus amyloliquefaciens*

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### Introduction

One of the major problems faced by metropolitan cities like Mumbai is garbage disposal. Mumbai city generates approximately six thousand tons of garbage daily. Out of which nirmalya constitutes of 15 tonnes. The nirmalya is been regularly disposed into the dumping grounds and water bodies causing land and water pollution. One of the methods for bioremediation is vermicomposting. Vermicomposting is a bioconversion, oxidation process of organic materials and involves a joint action of earthworms

and microorganisms which is widely being used for solid waste management (Manyuchi and Phiri, 2013). In this process, earthworms feed on the organic materials and convert it to vermicompost and vermiwash. Vermicompost has sweet and earthy pleasant smell like the smell of first rain (Kadam, 2004). Earthworms play a vital role in converting the organic matter to a bio-fertilizer. The epigeic earthworm *Eisenia foetida* is the most suitable species for vermicomposting as they have small size, short life

cycle and high rate of reproduction as well as high conversion of organic waste into compost (Nath et al., 2009; Chauhan and Singh, 2012). At the age of 6 weeks earthworms start laying cocoons (eggs). The main part of the digestive system in is anterior intestine that secretes different proteolytic enzymes for digestion of organic food particles and posterior intestine that absorbs nutrients. The earthworms aids in compost formation by acting as a turners, mixers, pathogen controllers, accelerators and aerators. One of the unique features of vermicompost is that during the process of conversion of various organic wastes by earthworms, many of the nutrients are changed to their available forms in order to make them easily utilizable by plants. Vermicomposts have higher level of available nutrients like nitrate or ammonium nitrogen, exchangeable phosphorous and soluble potassium, calcium and magnesium derived from the wastes (Buchanan et al., 1988). Edwards (1988) reported that vermicompost could promote early and vigorous growth of seedlings. Vermicompost has found to effectively enhance the root formation, elongation of stem and production of biomass, vegetables, ornamental plants etc. (Grappelli et al., 1985; Kale and Bano, 1986; Kale et al., 1987; Kale, 1998; Bano et al., 1993; Atiyeh et al., 2001b).

In the present work nirmalya (temple waste) was used as a substrate for vermicomposting and chemical analysis of vermicompost was carried out. Effect of vermicompost on plant growth was studied. The vermiwash was used to show presence of different types of bacteria that produce various types of enzymes.

## Materials and Methods

### Collection of substrate

The nirmalya was collected from a temple in South Mumbai using clean and dry plastic bags.

### Collection of earthworms

Earthworm species *Eisenia foetida* was purchased from local supplier Kalpataru, Mumbai.

### Setting up of a composting kit

In our work galvanized steel wire mesh kit of dimensions 20"×48"×33" (l×b×h) with a lid was constructed (figure 1A). The kit had proper aeration of minimum 1 cubic feet in volume. Trays of proper dimensions were kept below the kit to collect the drained water from kit. A green shade net was used to prevent the escape of earthworms from the kit and to

avoid predators from harming the earthworms. The shade net was stitched according to the size of the container (figure 1B).

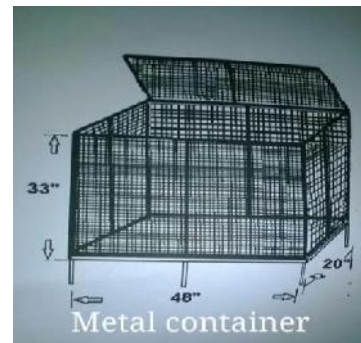


Figure 1A: Metal Container



Figure 1B: Metal Container set up

### Preparation of composting bed

The first layer consisted of sugarcane bagasse and coconut flax about 2"- 3" in height. The second layer was prepared by mixing fresh cowdung with water in 50:50 proportions (Gurav and Pathade, 2011) and was evenly spread on the basal layer. Thin slurry of garden soil was evenly spread on cowdung layer. Water was added to the kit to retain the moisture in the kit.

### Addition of nirmalya

After collection of flower waste from temples non-biodegradable part was removed by hand sorting and the biodegradable waste i.e garlands and flowers were segregated and shredded into small pieces. The segregated floral waste was air dried by spreading over paper for 48 hours. Initially three kg of freshly collected and finely chopped and dried nirmalya pieces were added into the kit. This system was kept without disturbance for a week. This process is called as precomposting.

### Addition of earthworms

In our work 500 grams of Epigeic species of earthworms *Eisenia foetida* were added to the kit. It is omnipresent with a world-wide distribution. It has good temperature tolerance and can live in organic wastes with different moisture contents. The pile containing nirmalya and earthworms was mixed at an interval of two days. Water was sprinkled on the top layer to ensure proper moisture. About one and a half kg of nirmalya was added thrice a week to the kit.

### Following precautions were taken during vermicomposting

Vermicompost kit was protected from direct sunlight. The moisture level was maintained well in the kit for good compost formation. Pests were avoided which might harm the earthworms. The kit was well covered to avoid the earthworms from escaping the kit. Sufficient aeration was provided by proper stirring of the compost pile to get rid of foul odour. A proper shed was built around the kit so that rain water doesn't enter the kit.

### Recovery of vermicompost

The processes of vermicomposting were carried out for a period of 90 days. The temperature of 30°C and 80% moisture content were maintained by sprinkling adequate quantity of water at frequent intervals. Vermicompost was obtained after 90days of incubation. After preparation of vermicompost, water was not added for 5 days to make the compost easy for shifting. The compost was collected in a separate container so that the earthworms settled at the bottom were reused for next batch of vermicomposting. The vermicompost obtained was brownish-black colour having a pleasant earthy smell. The prepared

vermicompost was packed in polythene bags and stored.

### Extraction of vermiwash

During the process of vermicomposting, drained water was collected as vermiwash which was used for further screening. The vermiwash was collected in trays and transferred into sterile glass bottles and stored in refrigerator at 4°C.

### Chemical analysis of vermicompost

The vermicompost was tested for concentrations of Organic Carbon (Walkley and Black method), Nitrogen (Micro Kjeldahl), Phosphorus (Olsen method), Potassium (Flame photometry), Zinc, Copper, Iron and Manganese (Atomic Absorption Spectrophotometer [AAS]). This analysis was done at Viva centre for Advanced Research and Development, Virar (W), Mumbai

### Study of microflora from vermiwash

This vermiwash was filtered using a muslin cloth to remove solid particles. The filtered vermiwash was then serially diluted with sterile phosphate buffered saline (pH7.2) and dilutions used for primary screening were  $10^{-1}$ ,  $10^{-2}$ ,  $10^{-3}$ ,  $10^{-4}$ ,  $10^{-5}$  and  $10^{-6}$ . From each dilution 0.1 ml was surface spread on sterile media such as Nutrient agar (for total bacterial count), Congo Red Yeast Extract Mannitol Agar (for detection of Rhizobium) and Ashby's Mannitol agar (for detection of Azotobacter) and incubated at 30°C for 24hrs. All the media were prepared using Hi Media manual (1998). Colonies on Nutrient agar were counted and cfu/ml was calculated. Different colonies were then picked up and were screened for various enzyme activities using the media as given in the Table 1 (Hi Media manual, 1998).

Table 1: Qualitative detection of Enzymes

MEDIA	ENZYME ACTIVITY
Sterile Starch agar plate	Amylase
Sterile Smith and Goodner's Gelatin agar plate	Gelatinase
Sterile Pikovskaya's agar plate	Phosphatase
Sterile Gorodkova's Tributyrin agar plate	Lipase
Sterile Milk agar plate	Caseinase
Sterile McBeth's cellulose agar plate	Cellulase

### Identification of the isolate

Identification was carried out on the basis of morphological, cultural and biochemical properties using Bergey's Manual of Bacteriology 8<sup>th</sup> Edition (1974). Further confirmation of the strain was done by 16s rRNA sequencing analysis (Yaazh Xenomics, Mumbai).

### Quantitative lipase assay

The colony obtained on Gorodkova's tributyrin agar medium showing large zone of clearance was enriched in nutrient broth at 30°C for 48 hours. Lipase activity was assayed by Para-nitro Phenyl Palmitate Assay which is further modified method of Winkler and Stuckmann (1979). The fermented broth was centrifuged at 10,000 rpm at 4°C for 20 minutes (Joshi et al., 2006). The supernatant obtained was used as a crude enzyme for lipase assay (Qamsari et al., 2011). This crude enzyme (0.75 ml) was mixed with 0.5mM of 4-nitrophenyl palmitate substrate prepared in isopropyl alcohol and 1.95 ml of 50mM Phosphate Buffer (pH7.2) incubated at 30°C for 30 minutes. After incubation the reaction mixture was kept in ice bath for 5 minutes to stop the reaction and 150µl of Triton X-100 was added to the mixture. It was centrifuged at 10,000 rpm for 25 minutes and the absorbance of the supernatant was recorded spectrophotometrically at 420 nm. The reaction mixture containing heat-inactivated crude enzyme (100°C for 10 min) instead of the active culture supernatant was used as a blank. The absorbance of the test supernatant against the blank was obtained and plotted on the standard graph of *p*-Nitrophenol (2-20µg/ml) to obtain the amount of substrate converted. One unit of lipase activity is the amount of lipase enzyme, which liberates 1 µmole of *p*-Nitrophenol from 4-Nitrophenylpalmitate as substrate per minute under standard assay conditions (Aruna and Khan, 2014).

### Quantitative protease assay

The colony obtained on skimmed milk agar medium showing large zone of clearance was selected as protease producers. Protease assay was carried out by Folin Lowry method (Lowry et al., 1951). The colony obtained on skimmed milk agar plate was enriched in nutrient broth with 1% casein flask and incubated at 30°C for 24 hours on a rotary shaker (1000rpm). The cells were then separated from broth by centrifugation at 5000rpm for 20 minutes. The supernatant obtained was considered as crude enzyme extract. Protease activity was measured using Caseinolytic assay with some modifications (Aruna et al., 2014). The enzyme extract (0.1ml) was reacted with 9ml of 1% casein prepared in phosphate buffer (pH-7) at 30°C for 20 minutes. After which, 1.5ml of Trichloro acetic acid (5% w/v) was added to arrest the reaction. After 10 minutes the reaction mixture was centrifuged at 5000 rpm for 15 minutes. Absorbance of the supernatant was measured by modified Folin- Ciocalteu method (Lowry et al., 1951), against inactive enzyme. A standard graph of concentration of standard tyrosine (10-100µg/ml) against absorbance at 660nm was plotted.

1 unit of Enzyme activity (unit/ml) =

$$\frac{\text{amount of tyrosine produced } (\mu\text{M})}{\text{Time of reaction (min)} \times \text{Volume of enzyme (ml)}}$$

### Pot culture studies using Vermicompost obtained from nirmalya

Small plastic pots with 1000gms of soil for control and 200gms of vermicompost plus 800gms of soil for test were used for pot culture studies. The two different plants (*Tagetes erecta* and *Solanum melongena*) seeds were purchased from Ratanshi's Agro-Hortitech Store, Mumbai. Daily 30 ml of tap water was added in each pot. Growth parameters like number of leaves, plant height, width and length of plant leaves were measured in (cm) using scale and recorded after 40 days.

Table 2: Different plants used for pot culture studies

Sr no	Common name	Botanical name
1	Marigold	<i>Tagetes erecta</i>
2	Brinjal	<i>Solanum melongena</i>

## Results and Discussion

Nirmalya was used as a substrate for vermicomposting (figure 2) using *Eisenia foetida* (Figure 3) as earthworm species. The temperature 30°C and moisture content (80%) were maintained by sprinkling adequate quantity of water at frequent intervals. The process of vermicomposting was carried out for a period of 90 days. Vermicompost harvested after 90 days of incubation was granular, dark brown in colour as shown in figure 4. In the vermicomposting bed, the first layer consisting of coconut flax and sugarcane bagasse, which is rich in cellulose, serves as a base for moisture retention. The second layer consisting of cowdung slurry acts as an inoculant which influences and accelerate organic waste breakdown. Nirmalya

which used as a substrate is a rich source of complex macromolecules which can be easily broken down by secretory enzymes of earthworms and contained most of the constituents favourable for the growth. The precomposting being thermophilic in nature prior to vermicomposting helped in pathogen and mass reduction (Nair et al., 2006; Gurav and Pathade, 2011). In our work, the period of vermicomposting using nirmalya was found to be 90 days. However, Singh et al. (2013), Gurav and Pathade (2013), Kohli and Hussain (2016) and Tiwari and Juneja (2016) have reported vermicomposting period using flower waste as 120 days, 30 days, 45 days and 50 days respectively. Vermicomposting period may vary between 25 to 252 days depending upon type of substrate used (Lim et al., 2016).



Fig 2: Fine pieces of Nirmalya. Fig 3: Earthworms (*Eisenia foetida*) Fig 4: Vermicompost

The Chemical analysis of vermicompost was carried out. The analysis report of soil and vermicompost is given in Table 3.

Table 3: Chemical analysis of vermicompost obtained from nirmalya waste

PARAMETERS	SOIL VALUES	VERMICOMPOST VALUES
pH	6.82	7.2
Potassium (%)	0.12	0.16
Organic carbon (%)	1.4	8.57
Total phosphorous (%)	0.8	0.5
Zinc(ppm)	1.44	3.9881
Copper (ppm)	8.18	1.166
Manganese(ppm)	17.68	11.2
Iron(ppm)	21.96	691.9
Nitrogen (%)	0.03	0.49
C:N ratio	46.66	17.489
C:P ratio	1.75	17

In our work the pH value of vermicompost was found to be 7.2. These results are in agreement with the results of Kohli and Hussain (2016) and Jain (2016) who reported that pH of floral vermicompost was 7.53 and 7.2 respectively. Similar results were also obtained from vermicompost from Municipal waste (Narkhede et al., 2011) and organic waste (Punde and Ganorkar, 2012; Chanda et al., 2011). Chakole and Jasutkar (2014), Singh et al. (2013), Gurav and Pathade (2011), Shouche et al. (2011) and Makhania and Upadhyay (2015) also reported that the pH of the vermicompost from nirmalaya or temple solid waste varies between 7.9 to 8.48 which is slightly higher than our studies. The lowering of pH in our studies might be due to the carbon dioxide and organic acids production by microbial activity during vermicomposting process (Haima and Hutha, 1986). The pH range of vermicompost from 6 to 8.5 is best suited to ensure compatibility with the most plants (Hogg et al., 2002). The pH of Vermicompost is reported to be substrate dependent and earthworms maintain the pH of vermicompost in the neutral range (Chakole and Jasutkar, 2014).

Vermicomposts have higher level of available nutrients like nitrate or ammonium nitrogen, exchangeable phosphorous, soluble potassium, calcium and magnesium derived from the wastes (Buchanan et al., 1988).

The concentration of the total potassium (TK) greatly increased in the vermicompost as compared to soil in our studies. The TK content of the vermicompost in the present study was found to be 0.16% which is in accordance with the results of Kale et al. (1995) and Jaybhaye and Bhalerao (2015) who reported the TK level 0.15% and 0.16% respectively. However, TK was reported in vermicompost prepared from temple waste 0.28% (Gurav and Pathade, 2013), 0.5% (Singh et al., 2013) and 0.81% (Jain, 2016). There are also other reports of TK value 1.4% (Narkhede et al., 2011) and 3% (Ansari and Rajpersaud, 2012) of vermicompost obtained from municipal waste and cow dung waste respectively. A TK value of vermicompost varies from 0.15% to 0.73% (Kohli and Hussain, 2016). Vermicomposting has been established as an effective process for recovering higher K from organic waste (Manna et al., 1997; 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). The change in the distribution of potassium between exchangeable and non exchangeable forms may lead to an increase in the potassium content in the

vermicompost. The earthworm processed the waste material which contained high concentration of exchangeable potassium, due to enhanced microbial activity during the vermicomposting process and it consequently enhanced the rate of mineralization (Achshah and Prabhu, 2013).

The vermicompost had 8.57% of total organic carbon (TOC) as compared to 1.4% of organic carbon content of soil. Vermicompost TOC value varies from 9.8 to 13.4% (Kohli and Hussain, 2016; Ahirwar and Hussain, 2015). However, the level of total organic carbon content in the vermicompost obtained from temple wastes was found to be 18.9 % (Singh et al., 2013), 28% (Gurav and Pathade, 2013), 16.34% (Chakole and Jasutkar, 2014) and 20.76% (Jain, 2016). There are also other reports of TOC value 26.41% and 25.12% from vegetable and paper waste respectively (Kapoor et al., 2015). The incorporation of floral waste vermicompost has been shown to increase organic carbon content in the soil (Nelson and Sommers, 1982). Increase in the level of total organic carbon may be due to the addition of earthworm's cast, which is rich in carbon or due to the presence of high amount of organic matter in waste (Kaviraj and Sharma, 2003) and feeding action of earthworms and decomposition by microbes. The combined process brings about carbon loss from substrates in the form of carbon dioxide. The microbial respiration may lead to rapid carbon loss through CO<sub>2</sub> production and also, digestion of carbohydrates, lignin, cellulose and other polysaccharides from the substrates by inoculated earthworms may cause carbon reduction during the decomposition of organic waste (Kaushik and Garg, 2003; Suthar, 2007; Venkatesh and Eevera, 2008).

Soluble Phosphorus of vermicompost value varies from 0.9-1.02% (Kohli and Hussain, 2016; Chanda et al., 2011). The concentration of soluble phosphorous was found to be 0.5% in the vermicompost in the current study and 0.8% in the soil. These results are in accordance with the results reported by Gurav and Pathade (2011). Similar result was also reported from vermicompost obtained from grass waste where phosphorous level was 0.2-0.6% (Ansari and Rajpersaud, 2012; Jaybhaye and Bhalerao, 2015). However, other results reported the higher phosphorous level 1.3 % in the vermicompost produced from temple waste (Singh et al., 2013) and other organic wastes (Mistry et al., 2015). The conversion of unavailable forms of phosphorus to easily available forms for plants takes place during vermicomposting (Ghosh et al., 1999). The decrease of soluble reactive phosphorous (SRP) can be

explained by the precipitation of soluble phosphorous with other cations making the SRP less soluble (Kiefer, 2012) or because SRP is very easily taken up by the organisms present within the vermicompost (Holtan et al., 1988). The activity of phosphatase is responsible for solubilization of insoluble phosphate.

The micro-organisms in the vermiproducts play a significant role in altering the soil micronutrient content (Manyuchi et al., 2013). Zinc exists in the soil as  $Zn^{2+}$ . Zinc content of vermicompost value varies between 4.2 to 11ppm (Kohli and Hussain, 2016). In current studies the level of zinc in vermicompost was found to be 3.988ppm and 1.44ppm in the soil. However, zinc level 12.5ppm in the vermicompost obtained from nirmalya was reported by Gurav and Pathade (2011). The Zinc concentration in the vermicompost is corresponding to the results of Geiklooi and Shirmohammadi (2013). There is also report of 1.47ppm of Zinc in vermicompost obtained from kitchen waste and cow dung (Jaybhaye and Bhalerao, 2015). Their investigation reveals the effect of vermicompost on improving the zinc and iron deficiencies of soil. Zinc is a microelement involved in auxins, carbohydrate, phosphate, proteins, RNA metabolism and ribosome formation in plants. Zinc is also the essential regulatory cofactor of variety of enzymes and hence is required for many metabolic processes in plants (Mengel and Kirkby, 1982). Increasing the vermicompost quantity applied increased the soil zinc content to more than 1.6ppm (Manyuchi et al., 2013).

Copper content of vermicompost value varies between 2.6ppm and 4.8ppm (Kohli and Hussain, 2016). The vermicompost had 8.18ppm of copper content as compared to 1.16 ppm copper content of soil. However, higher level of copper 30ppm in the vermicompost produced from nirmalya was reported by Gurav and Pathade, (2011). Values of Copper content of vermicompost such as 0.54ppm (Jaybhaye and Bhalerao, 2015) and 5ppm (Kaur et al. 2015) are also reported. Copper (Cu) attributes to many physiological processes in plants such as photosynthesis, respiration, carbohydrate distribution, nitrogen metabolism, seed production and disease resistance (Kabata-Pendias and Pendias, 2001). However, high level of copper can be responsible for phytotoxicity and also leads to reduction in seed germination, plant growth and iron uptake (Paz-Ferreiro et al., 2014).

The vermicompost had iron content of 691 ppm as compared to the 21.96 ppm iron content of tested soil.

Similar result having high levels of iron content 860ppm in the vermicompost obtained from nirmalya was reported by (Gurav and Pathade, 2011). Usually vermicompost has iron content which shows variation from 205ppm to 1133ppm (Kohli and Hussain, 2016; Jaybhaye and Bhalerao, 2015). In another study the iron level was reported as 175ppm and 146.5 of vermicompost and farmyard manure respectively (Kaur et al., 2015). The presence of enzymes and co-factors in the earthworm gut increase the iron content in the vermicompost. If high concentration of iron is present, Iron replaces Manganese (Mn) in organo-mineral complexes, and the released Mn is precipitated thus reducing the availability of Mn (Smith et al., 2001). The mineralization of partially digested worm fecal by fungi and bacteria and their action in the foregut resulted in high levels of trace elements like Zinc and Iron in vermicompost (Vasanthi et al., 2013).

Manganese content of vermicompost was 11.2ppm while soil showed 17.68ppm content. Similar results were obtained from vermicompost (Yadav, 2015; Jaybhaye and Bhalerao, 2015). However, Manganese content of vermicompost obtained from temple waste was 95ppm (Gurav and Pathade, 2011). Generally manganese content of vermicompost varies between 10.5ppm and 20.38ppm (Kohli and Hussain, 2016). Manganese exists in the soil as  $Mn^{2+}$ . Increase in the vermicompost quantity in the soil results in increased manganese content by more than 200ppm (Manyuchi et al., 2013).

The concentration of nitrogen in the vermicompost was found to be 0.49% and 0.03% of the soil. These results are in agreement with the results of Borah et al. (2007) who reported the nitrogen content as 0.38%. However, higher level of nitrogen was reported in vermicompost prepared from temple waste 0.91% by Chakole and Jasutkar (2014) and 1.58% by Gurav and Pathade (2011) while other organic wastes showed nitrogen content 1.32% (Punde and Ganorkar, 2012) and 0.805% (Jaybhaye and Bhalerao, 2015). Generally nitrogen content of vermicompost varies between 0.51% and 1.61% (Kohli and Hussain, 2016; Chanda et al., 2011). Increase in nitrogen content is due to the fact that earthworms enhanced the nitrogen cycle which attributed to the increased levels of nitrogen in vermicompost. The increased nitrogen content may be due to nitrogenous metabolic products of earthworms (Umamaheswari and Vijayalakshmi, 2003). Similar result was reported by Hand et al. (1988) who found that *Eisenia foetida* in cow slurry increased the nitrogen content of the substrate.

The increase in amounts of nitrogen and decrease in carbon content (reduced C: N ratio) is an indication of increased mineralization of the elements due to enhanced microbial and enzymatic activities in earthworm gut (Parthasarathi and Ranganathan, 2000). The C:N ratio of vermicompost in the current studies was found to be 17.489 as compared to 46.6 of soil. These results are in accordance with the results of Mistry et al. (2015). Similar results of C: N ratio 15.54 and 16.03 in the vermicompost obtained from vegetable wastes are present (Kapoor et al., 2015). Vermicompost obtained from flower waste has C: N ratio 12.3 (Kohli and Hussain, 2016) while temple waste vermicompost has C: N ratio 19.36 (Singh et al., 2013), 17.38 (Chakole and Jasutkar, 2014) and 21.55 (Jain, 2016). C: N ratio is one of the most widely used indicators of vermicompost maturation, which declines during the vermicomposting process (Kale, 1998; Gupta and Garg, 2008; Suther, 2008). Decrease in the C: N ratio in vermicompost to less than 20 indicates an advanced degree of organic matter stabilization and mineralization (Senesi, 1989).

The C: P ratio was found to be 17.5:1 in the vermicompost in our studies. C: P ratio obtained in our work is in accordance to the results of Singh et al. (2013) who reported the C: P ratio as 15:1 from temple waste. The ratio C: P of 15:1 in vermicompost indicates stabilization and maturity of organic wastes

which is beneficial for better assimilation by plants (Edwards and Bohlen, 1996).

Six liters of vermiwash was collected from the kit after completion of vermicomposting process and it was found as dark brown in colour and odorless. The obtained vermiwash was analysed for beneficial microorganisms. The total bacterial count in the vermiwash was found to be  $3 \times 10^9$  cells/ml. This result runs parallel to that of Giraddi (2007) who reported the total bacterial count in vermicompost as  $1.13 \times 10^8$  cells /ml. Devi et al. (2009) also reported presence of bacteria, fungi and actinomycetes during vermicomposting with maximum number of  $126 \times 10^6$ ,  $28 \times 10^4$  and  $93 \times 10^5$  CFU /g of sample respectively.

The microflora of vermiwash contained *Azotobacter* and *Rhizobium* which are nitrogen fixing bacteria. *Rhizobia* appeared as white coloured colonies on Congo Red Yeast Extract Mannitol agar (CRYEMA) plate as shown in figure 5A while *Azotobacter* has grown on Ashby's Mannitol agar plate (figure 5B). Zambare et al. (2008) reported that vermiwash contains nitrogen fixing bacteria like *Azotobacter* sp. and *Rhizobium* sp., which make the inorganic nitrogen and amino acids available to plants. The availability of Nitrogen was also increased indicating vermicompost may attribute the significant increase in nitrogen of the soil by using floral vermicompost (Parmelle and Crossley, 1988; Tiwari et al., 1989; Scheu, 1993).

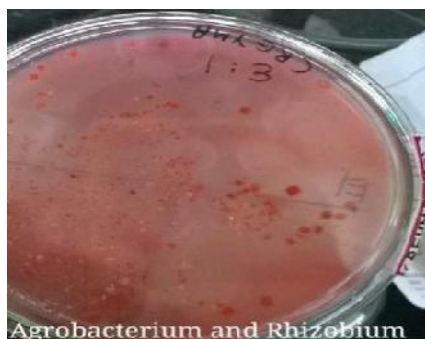


Figure 5A: CRYEMA plate showing growth of *Rhizobium*.

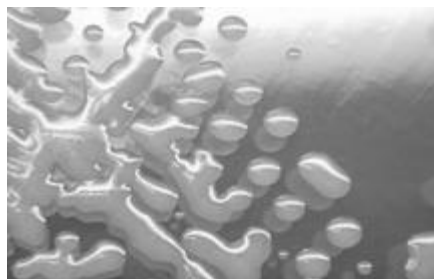


Figure 5B: Ashby's Mannitol agar plate showing growth of *Azotobacter* spp.



Qualitative detection of the following enzymes like lipase, gelatinase, amylase, cellulase and caseinase produced by different isolates from vermiwash was carried out as shown in figures 6, 7, 8, 9 and 10 respectively. Zambare et al. (2008) have reported that vermiwash contains various enzymes cocktail such as protease, amylase, urease, phosphatase, caseinase and gelatinase. In vermicompost, the maximum enzyme activities (cellulase, amylase, invertase, protease and urease) were observed by Devi et al. (2009). Presence of proteases in soil helps in seed germination while amylases help for availability of simple carbon source for enhancement of plant growth and productivity. Cellulases play an important role in global recycling

of the most abundant polymer (cellulose) in nature. Amylases and cellulases are responsible for rate of decomposition process in soil. Hydrolytic enzymes such as cellulase, lipase and proteases are responsible for complete decomposition and humus formation from organic matter (Kiss, et al., 2000, Marialigeti, 1979). Lipases are probably rate controlling during germination and lipase activity is high during seed germination (Brockhoff and Jensen, 1974). Soil borne microflora is essential for the growth of plants because organic nitrogenous compounds are decomposed and mineralized by different enzymes produced by nitrogen fixing and phosphate solubilizing bacteria (Chaudhary, 2005).



Figure 6: Gorodkova's Tributyrin agar plate showing Lipase activity



Figure 7: Smith and Goodner's Gelatin agar plate showing Gelatinase activity.



Figure 8: Starch agar plate showing Amylase activity.



Figure 9: Mc Beth agar plate showing Cellulase activity

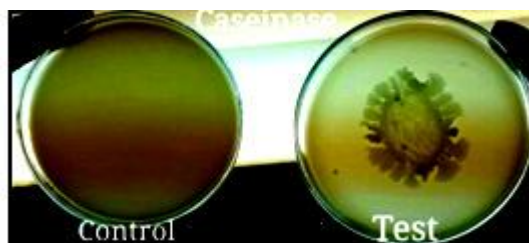


Figure 10: Milk agar plate showing Caseinase activity.

One of the isolates was characterized as aerobic, gram positive and rod shaped bacteria. From the biochemical tests, it was concluded that the isolate belongs to the family of *Bacillus* (Bergey's Manual of Bacteriology 8<sup>th</sup> edition, 1974). It was identified as *Bacillus amyloliquefaciens* HY10 by carrying out 16s rRNA gene sequence analysis. Quantitative estimations of the protease and lipase enzymes produced by *Bacillus amyloliquefaciens* HY10 were carried out as shown in Table 4. *Bacillus*

*amyloliquefaciens* HY10 considered as growth-promoting rhizobacteria and has the ability to quickly colonize roots. It is also used to fight some plant root pathogens in agriculture, aquaculture and hydroponics (George et al., 1995; Saengsanga et al., 2016). There are several studies which indicate presence of different types of microorganisms including *Bacillus* species in the vermicompost (Vivas et al., 2009; Vaz-Moreira et al., 2008; Pathma and Sakhivel, 2012 )

Table 4: Quantitative estimation of enzymes produces by *Bacillus amyloliquefaciens* HY10

ENZYMES	ENZYME ACTIVITY (Units/ml)	SPECIFIC ACTIVITY (U mg <sup>-1</sup> Protein)
Protease	32.53	367.98
Lipase	3.177	47.91

Vermicompost and vermiwash used as organic manure has a property of binding with minerals in the form of humus colloids and clay, promoting stable soil aggregates resulting in better aeration and desired porosity to sustain plant vigour (Haynes, 1986). The vermicompost obtained was tested for its effectiveness by monitoring different plant factors such as number of leaves, leaf length, leaf width and plant height for Brinjal plant after an interval of 40 days (*Solanum melongena*) as shown in table 5 and figure 11. The similar growth pattern was also observed for the Marigold Plant (*Tagetes erecta*), as shown in Table 6

and figure 12 where plant of the test as compared to the control used in the studies showed good enhancement of growth. The test was supplemented with vermicompost while control was without supplementation. Similar experimental studies were carried out on brinjal plants by many scientists (Lalitha et al., 2000; Raviv et al., 1998; Singh et al., 1998; Shivsubramanian and Ganeshkumar, 2004; Sailaja et al., 2013; Kashem et al., 2015; Jaybhaye and Bhalerao, 2015) who reported the better growth of plants and higher yield by the application of vermicompost. Vermicompost also has a significant

positive influence on seed germination and seedling vigor (Atiyeh et al., 2001a, 2002; Suthar *et al.* 2005; Arguello et al., 2006; Alam et al., 2007; Ansari, 2008; Gupta et al., 2008; Peyvast et al., 2008; Premsekhar and Rajashree, 2009; Suthar, 2009; Chanda et al., 2011). Flowering capacity, height of plant, breadth and length of leaves are found more in vermicompost supplemented tests as compared to controls (Yadav et al., 2015). Earthworms stimulate microbial activities and metabolism and also influence microbial populations. As a consequence more available nutrients and microbial metabolites are released into the soil (Tomati et al., 1988). Use of vermicompost is

effective for improving soil fertility and it contains most of the nutrients in plant available form such as nitrates, phosphates, exchangeable calcium and soluble potassium (Aggelides and Londra, 1999; Mascolo et al., 1999; Albiach et al., 2000; Marinari et al., 2000; Sailajakumari and Ushakumari, 2002; Arancon et al., 2006; Prabha et al., 2007; Azarmi et al., 2008). Vermicomposting contains plant hormones like Auxin and gibberellins and enzymes which believed to stimulate plant growth and discourage plant pathogens (Businelli et al., 1984; Tomati et al., 1988).

Table 5: Pot culture studies for Brinjal 9 (*Solanum melongena*) plant after 40 days.

PARAMETERS	SOIL(control)	SOIL +VERMICOMPOST(test)
Number of leaves	23	35
Leaf length	6cm	12cm
Leaf width	4cm	7.5cm
Plant height	22cm	35cm



Figure 11: Effect of vermicompost on Growth of Brinjal (*Solanum melongena*) plant a- Control (Only soil); b- Test (soil + vermicompost)

Table 5: Pot culture studies for Marigold plant (*Tagetes erecta*) after 40 days.

PARAMETERS	SOIL(control)	SOIL+ VERMICOMPOST(test)
Number of leaves	33	76
Leaf length	1.5cm	5cm
Leaf width	1cm	2cm
Plant height	10cm	24cm



Figure 12: Effect of vermicompost on Marigold Plant (*Tagetes erecta*) a- Control (Only soil); b- Test (soil + vermicompost)

## Conclusion

Vermicomposting is a natural process which not only gives a solution for waste management but also provides with nutrient rich compost which can be used for any kind of plantation. Vermicompost increases soil fertility as it consists of nutrients like phosphorus, nitrogen, carbon, zinc, copper, manganese, iron, etc. various enzymes and plant growth hormones like gibberellins, auxins and cytokinins and it also decreases the levels of organic carbon, C/N ratio and pH through vermic-activity in the soil. The nutrient content of vermicompost revealed that Nirmalya is a suitable substrate for vermicomposting. Hence, this eco-friendly method can be extended for all temple and alike organic wastes for analyzing the effectiveness of vermicompost. It helped to reduce volume of temple flower waste, but also generate additional revenue. Thus vermicompost technology can be successfully applied in temples as a solid waste management strategy with flowers as the major organic waste.

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