Effect of vermicomposting using *Eisenia foetida* in enhancing vermi degradation and optimization of fly ash with cow dung for different proportion of waste

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Abstract

Fly ash is conveyed of coal burning in thermal power plants makes a couple of common contamination issues and it needs considerable limit zone to keep it out of the earth. Fly ash management remains a noteworthy worry in the developing nations. On opposite the material has extraordinary potential for valuable use in horticulture. Vermicomposting is an extraordinary procedure for reducing the lethal overwhelming metals in fly powder through the activity of earthworms. In this study we assessed the possibility of subjecting the fly ash with flower waste for vermicomposting. Fly ash was mixed with Cow along with flower waste dung in 1:3:1, 3:1:1 and 2:2:1ratio and was incubated with *Eisenia fetida* for 60 days. The concentrations of nutrients (Phosphorus) were found to increase and EC, Total organic matter and total organic carbon were found to decrease in the earthworm-treated series of fly ash cow dung combinations compared with the control. In this study the bacterial strains were isolated from the vermicompost and also their enzyme activities, protein profile, antioxidant assay was performed. Among different combinations of fly ash, cow dung and flower waste, nutrient content was significantly higher in the 1:3:1 fly ash to cow dung along with flower waste treatment compared with the other treatments.

Keywords: Fly Ash, Eco friendly products, Eisenia foetida.

Introduction

Fly ash is the fine buildup caught from pipe depletes when coal is scorched in power stations. With the reliably expanding number of coal-terminated plants, the vast-scale generation of fly ash is making intense waste transfer issues in various parts of the world. The measure of ash created every year in India was around 90 million tons amid 1995 and is probably going to 140 million tons in 2020. Composting is one of the most encouraging approaches to reuse the wastes produced from power plants, as the procedure decreases the volume, and balances out of the waste. The high natural matter content in the fertilizer items likewise preserves soil richness. The appropriate change of parameters, for example, moisture, oxygen level, and temperature amid the composting process will decide the best possible working just as the attributes of the final result were obtained. Legitimate condition ought to be accommodated the development of microorganisms as well as the earthworm. The expansion of fly ash to the treating the soil procedure has no unfavorable impact on either C: N ratio proportion or microbial populace (Sunita Gaind and Gaur, 2003).

Vermicomposting is an appropriate innovation for decay of various kinds of natural waste into value included material (Payal et al., 2006). Likewise municipal solid waste can be exposed to vermicompost, which can yield high nutritive esteem (Kaviraj and Satyawati Sharma, 2003). Earthworms use the waste substrate and upgrade the rate of decay of the natural matter, prompting a treating the soil impact through which unsterilized natural matter ends up settled. The organic movement of earthworms gives supplements rich vermicomposting to plant development hence encouraging the exchange of supplements to plants. Another significant final result of the vermicomposting process is the vermiwash. It is generally amazing manure, development promoter and helps prompting blooming and natural product in higher plants. This cans even assistance plants to dispose of irritations and illnesses.

The limits of earthworms for the period of the time spent vermicomposting of waste are a physical and biochemical framework. The physical procedure incorporate substrate air circulation, blending as well as actual grinding while the biochemical procedure is impacted by microbial deterioration of substrate in the digestive tract of worms different analysis have exhibited that vermicomposting of natural waste quickens natural substance vary (James Frederickson et al., 1997) and gives chelating and phyto hormonal parts which have a high substance of microbial matter (Arancon et al., 2005) and balanced out humic substances. There has been an increasing development to decrease the rate of inorganic fertilizer functions to soil by using soil complements even more beneficially and by the delayed utilization of natural matter. Natural fertilizer is a significant source for keeping up soil fertility. Support of soil natural matter for continued soil profitability requires the contribution of natural fertilizer.

Currently there has been more accentuation on utilization of natural fertilizer everywhere throughout the world. The utilization of natural squanders in the application to farmlands has expanded throughout the years, since the utilization adds to the transfer of squanders and enhances the protection of the environment. The high natural matter content in the fertilizer product likewise safeguards soil richness. Among different wellsprings of natural issue, vermicomposts have been perceived as having significant potential as soil corrections. So vermicomposting procedure has been embraced broadly for natural cultivating.

Material and methods

Collection of materials:

Fly ash for this study was collected from Neyveli Lignite Corporation (NLC) Thermal Power Plants is located in Neyveli, TamilNadu, India. After collection the dry ash is thoroughly mixed and stored in plastic lined containers at room temperature before use. The earthworm species (*Eisenia foetida*) was collected manually from the vermicompost field which is located in Periyar Maniammai University, Thanjavur, India. Fresh cow dung is collected from a cow farm near the university campus.

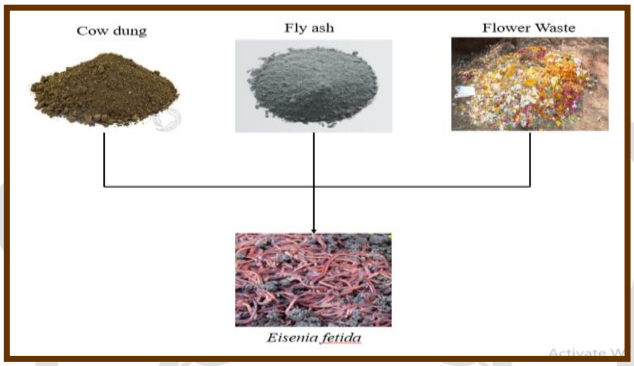


Fig: 1 Wastes subjected for vermicomposting

Table:1 Waste subjected for vermicomposting

S.No	Samples	Ratio of the sample	Weight of the sample (g)
1	Ash waste + Cow Dung + Flower Waste	1: 3: 1	100 + 300 + 100 (500g)
2	Ash waste + Cow Dung + Flower Waste	3:1:1	300 + 100 + 100 (500g)
3	Ash waste + Cow Dung + Flower Waste	2:2:1	200 + 200 + 100 (500g)

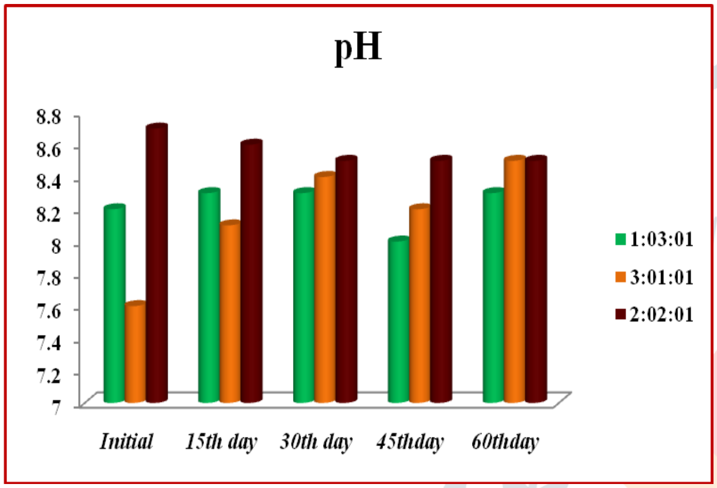
Earthworms culture:

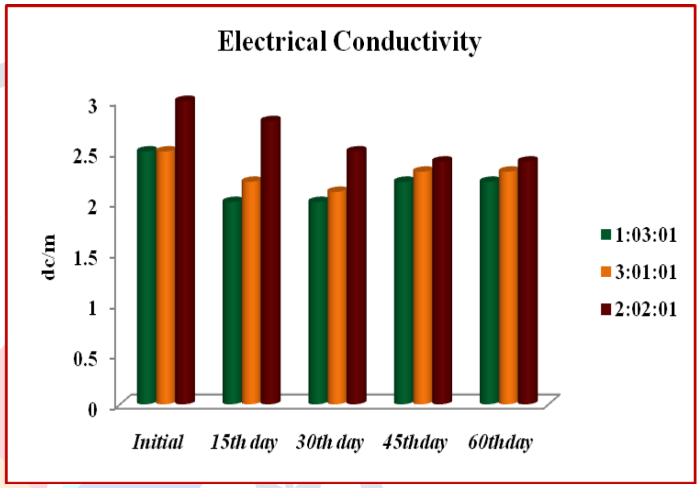
The culture of earthworms (*Eisenia foetida*) was maintained under laboratory conditions by using cow dung as a culturing material. The worm's culture was needed for time to time use of earthworms for research work.

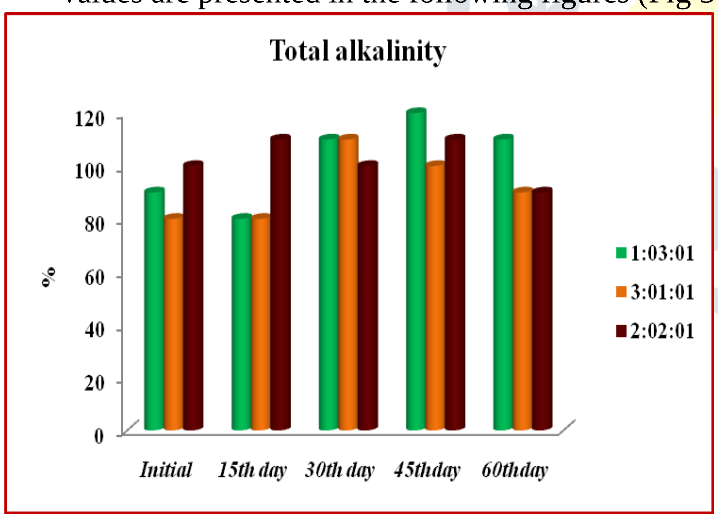
Different ratios of fly ash (FA), cow dung (CD) and flower waste (FW), FA + CD+ FW (1:3:1), FA+CD +, FW (3:1:1) , FA + CD+ FW (2:2:1) were used for the study. Tandon, (1993) methods followed by physico-chemical parameters were analyzed. FTIR were analyzed by Rajiv *et al.*, (2013), X-ray diffraction patterns of the sample were analyzed using XRD analyzer (Sartaj, 2014). Genomic DNA were determined using by Broderick *et al.*, (2004), PCR amplification was estimated by Vivas *et al.*, (2009), Antioxidant assay was estimated by Song et al., *(*2010), Enzyme activity were estimated Kim *et al., (*2001).

RESULTS AND DISCUSSION

Vermicomposting is a dynamic process affected by a large number of environmental and biological factors. Change in any of these components significantly influences the nature of fertilizer just as the time required for composting. The vermicompost was prepared from the mixture containing cow dung and flower waste different quantities of fly ash waste, along with the earthworm population. The vermicompost was analyzed for the parameters such as pH, EC, alkalinity, TOC, TOM, Total nitrogen and Phosphorous. The values are presented in the following figures (Fig 3-9)







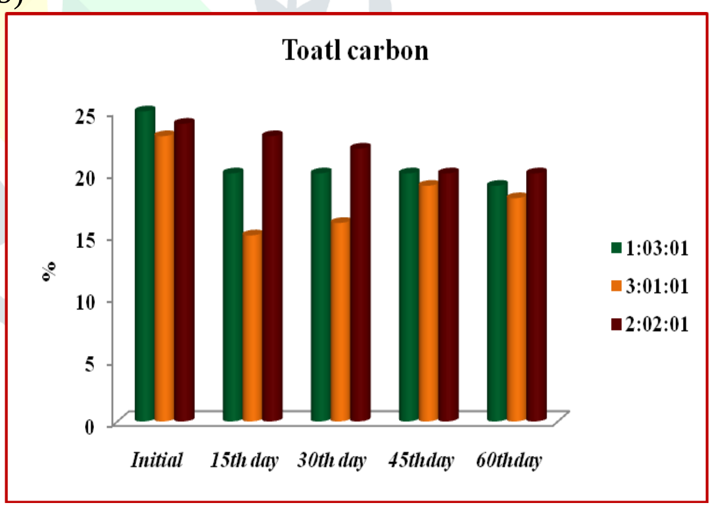


Figure: 3 pH **Figure :4 Elcetrical conductivit**

Figure : 5 Total alkalinity

Figure:6 Toatl carbon

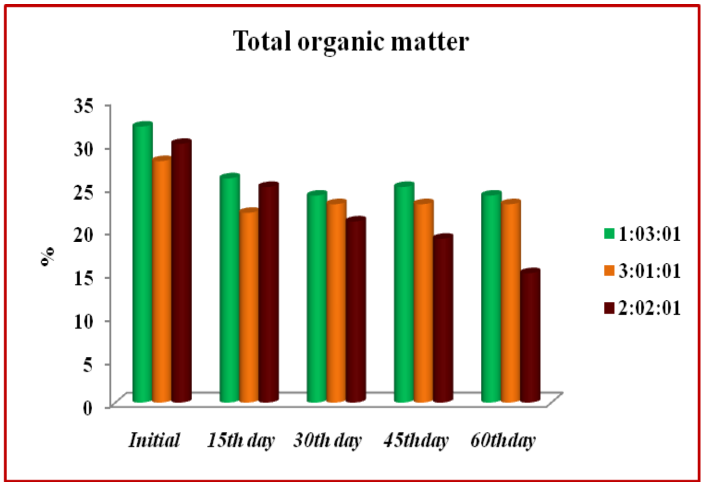


Figure : 7 Total organic matter

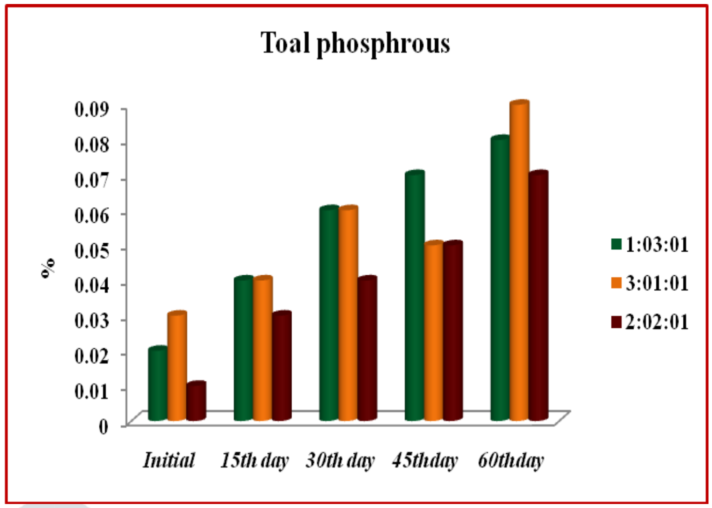


Figure: 8 Phosphorus

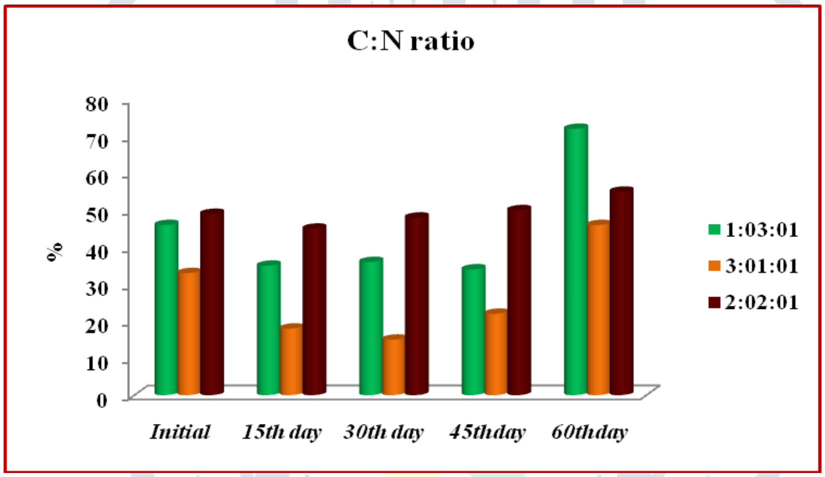


Figure: 9 C: N ratio

Variation of pH

The pH is one of the most frequent parameters used to characterize the vermicompost quality. As evident from Figure.3there were slight changes in the pH (Initial, 15^{th}, 30^{th}, $45^{th}$and 60^{th} days) values in 3 different ratios. The pH was (1:3:1), 8.2, 8.72, 8.3, 8.36 and 8.2. (3:1:1)7.9, 8.15, 8.42, 8.20 and 8.35 and (2:2:1)8.51, 8.6, 8.42, 8.36 and 8.23.The pH shift has been reported due to mineralization of nitrogen and phosphorus into nitrites/ nitrates and orthophosphoric acid (Ndegwa*et al.*, 2000).

Variation in EC

The EC values has decreased significantly in final vermicompost and was as follows in different ratios of wastes: (1:3:1)2.53 ms/cm,2.11 ms/cm, 1.96ms/cm, 1.76ms/cm and 1.68ms/cm. (3:1:1) 2.51 ms/cm, 2.23 ms/cm, 2.05 ms/cm, 1.94 ms/cm and 1.87ms/cm and(2:2:1)3.15ms/cm, 2.76ms/cm, 2.45ms/cm, 2.05ms/cm and

1.96ms/cm. The decrease in EC is due to loss of organic matter and release of different mineral salts in available forms such as phosphate, ammonium, potassium, etc (Kaviraj *et al.,* 2003).

Variation in Alkalinity

Alkalinity level has shown slight changes during the Ash waste in final vermicompost and was as follows in different ratios of wastes: (1:3:1) 90 mg/l,80 mg/l,110 mg/l, 120 mg/l and 100 mg/l. (3:1:1) 80 mg/l,80 mg/l, 110 mg/l, 100mg/land 90 mg/l and(2:2:1)100 mg/l,110 mg/l,100 mg/l,110 mg/l and 90 mg/l.

Variation in TOC

The reduction of TOC content was observed in the vermicompost. In this study, Figure.6 shows that the TOC has highly reduced during vermicomposting (1:3:1) 25.7%, (3:1:1) 21.6 % and (2:2:1)20.8 %treatments respectively. The reduction was higher in (1:3:1) 25.7 % vermicompost. The reduction in TOC during vermicomposting was also reported by Pattnaik and Reddy (2010). The author stated that during the decomposition process loss of carbon in the form of carbon dioxide was attributed to the earthworm and the microbial respiration. Our results showed that the maximum reduction of 25.7 % in (1:3:1) ratio vermicomposting, which may be due to the presence of microbial consortia.

Variation in TOM

Many wastes have been found to be readily decomposed by soil microbes. The main product of decomposition is carbon dioxide and water. Thus the decomposition of organic matter reduces the amount of TOM but leaves the compost enriched with nitrogen. The reductions in TOM were in ratios (1:3:1) 25.9 %, (3:1:1) 21.4 % and (2:2:1) 19.2%treatments respectively. The reduction was higher in (1:4) 25.9 % vermicompost for fly ash waste.

Variation in Total phosphorus

During vermicomposting process the Total phosphorus content in the feed mixture get significantly increased. Data showed in Figure.9 revealed that the amount of Total phosphorus got increased up to (1:3:1) 16 %, (3:1:1) 14 % and (2:2:1) 15.2 %, treatments respectively. The increase was higher in (1:4) 19 % vermicompost for fly ash waste. The increased in the Total phosphorus is caused by the activity of earthworms and phosphate solubilizing microbes during mineralization and mobilization of phosphorus (Ravindran *et al.*, 2015).

FTIR Analysis for Vermicompost:

The vermicompost prepared were subjected to FTIR analysis and the results are shown in fig 10 and 11.

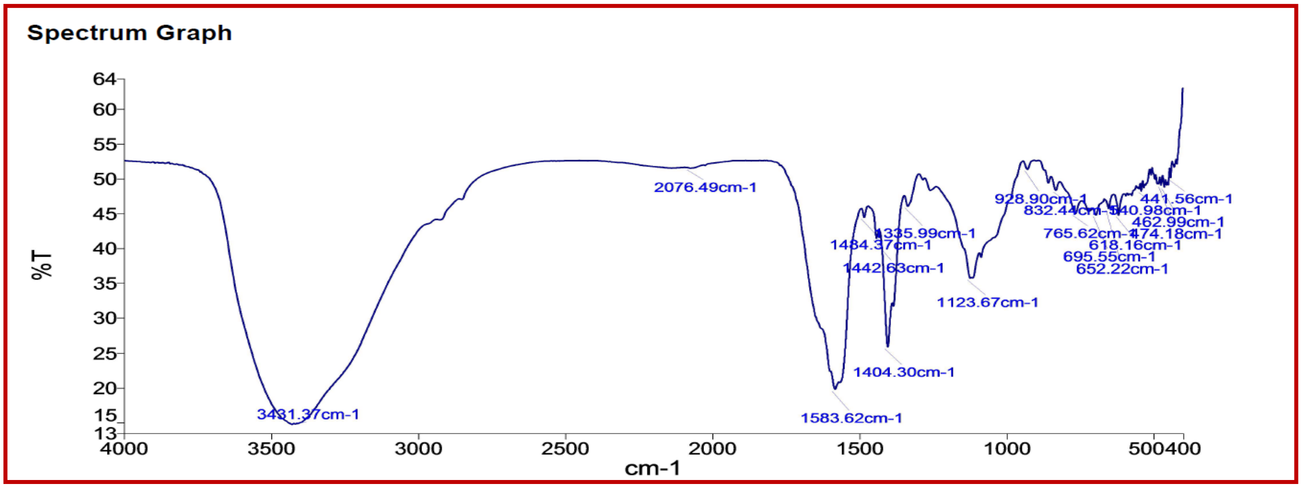


Figure: 10 FTIR pattern of the Initial Vermicomposting process

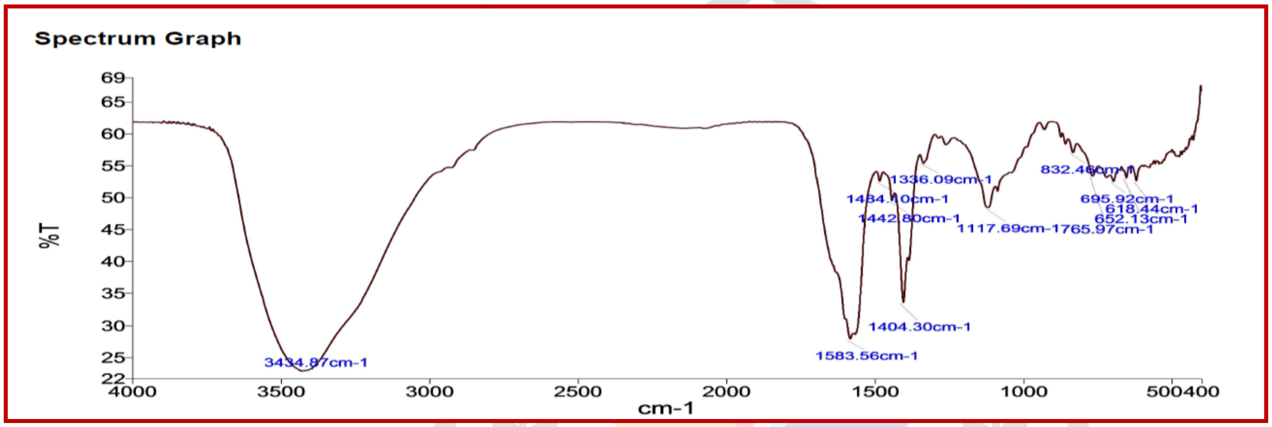


Figure: 11 FTIR pattern of the Final Vermicomposting process

FTIR analysis provides the information about the presence or absence of functional group which also indicates the degradation or stabilization process (Ravindran *et al.*, 2008). FTIR spectra of initial and final stage of Ash waste vermicompost samples are shown in fig (10 & 11). By comparing the initial and final stage there is a strong hydrogen bond due to -OH stretch was observed at 3431.78cm^{-1}. The band at 1123.67 cm^{-1} and 1117.69 cm^{-1} absorbed for lignin were also observed. While comparing the initial and final stage of vermicompost produced there is a slight reduction observed in previously discussed band. This proves the partial mineralization of cellulosic material. Hence the result confirms the reduction of aromatic structure, polypeptides and polysaccharides in the vermicompost prepared using Ash waste.

X – ray Diffraction analysis:

The vermicompost prepared were subjected to XRD analysis and the results are shown in fig. 12.

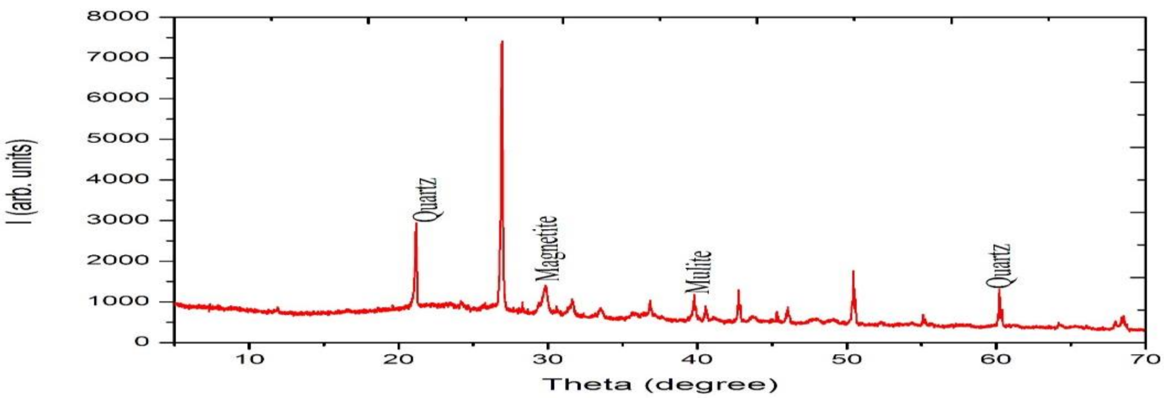


Figure: 12 XRD pattern of the Vermicomposting process

X ray diffraction study is used to study the crystallographic nature of fly ash waste present in the substrate and in the product. Fig.12 Hence the XRD results indicates that the Quartz peak at 60.9 ϴ, Multi peak at 40 ϴ and Magnetite peak at 31ϴ present in the fly ash waste.

Isolation of Genomic DNA and 16s rRNA

PCRAmplification

The Genomic DNA was further subjected to PCR amplification. Figure. 13 shows the 16S rRNA profile of the bacterial strains present invermicompost.

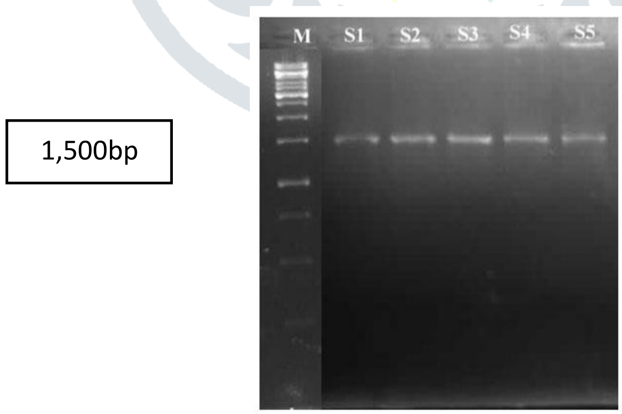


Figure: 13 16S rRNA profile of PCRAmplification (S1– S5) present in the vermicompost

Enzyme activity of the bacterial colonies isolated from the vermicompost

The isolated bacterial strains were subjected to Enzyme activity such as Protease, Amylase, and Cellulase results are as following figures (14 - 16).

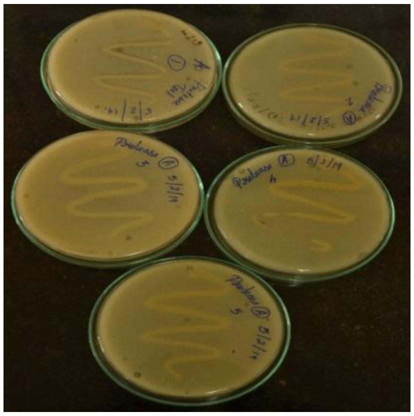


Figure: 14 Isolation of Protease producing bacteria from fly Ash vermicompost

Figure: 15 Isolation of Amylase producing bacteria from fly Ash vermicompost

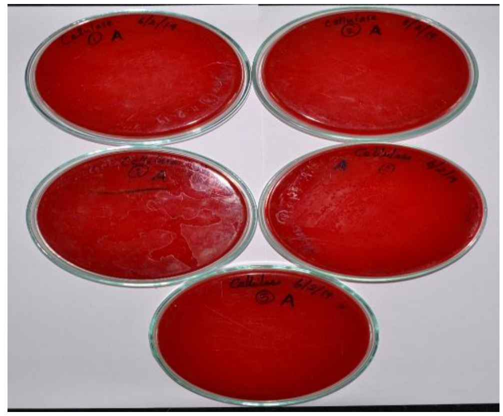


Figure: 16 Isolation of Cellulase producing bacteria from fly Ash vermicompost

Table: 2 Qualitative enzyme assay of the isolated bacterial cultures for amylase, protease, lipase and cellulaseactivity

Enzymes	Stain 1	Stain 2	Stain 3	Stain 4	Stain 5
Amylase	Positive	Positive	Positive	Positive	Positive
Cellulase	Negative	Negative	Negative	Negative	Negative
Lipase	Negative	Negative	Negative	Negative	Negative
Protease	Positive	Negative	Negative	Negative	Negative

The bacterial colonies isolated from the Vermicompost were subjected for enzyme analysis using starch agar medium for amylase; skim milk agar medium for protease; LB tributyrene for lipase and Czapek mineral salt agar for cellulose. The plates were observed for the zone of clearance called halo. The appearance of clear halos around the bacterial strains confirmed the respective enzyme production of the strains. The halo diameter was measured using the ruler for the comparison of the enzyme production among the isolates and are represented in figure. 14 – 16.

Antioxidativeassay

The biological responses earthworm (*Eisenia fetida*) samples were analyzed for the Antioxidative assays such as super oxide dismutase (SOD), Glutathione Peroxidase (GPx), Catalase (CAT), Glutathione (GSH) and Glutathione S-Transferase (GST) utilized for vermicomposting of Fly ash waste. Values were expressed as mean ± SD (N=6). Bar with different alphabets are significantly different from each other and with same alphabets have insignificant changes (P<0.05). The results are as follows figure 17-21

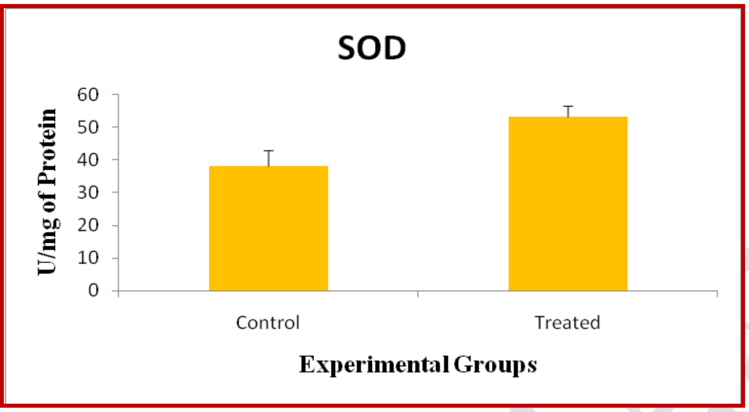


Figure: 17 Biochemical responses SOD activity

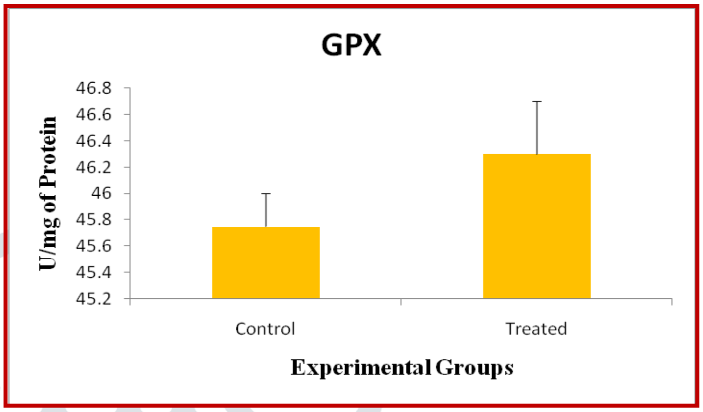


Figure: 18 Biochemical responses GPX activity

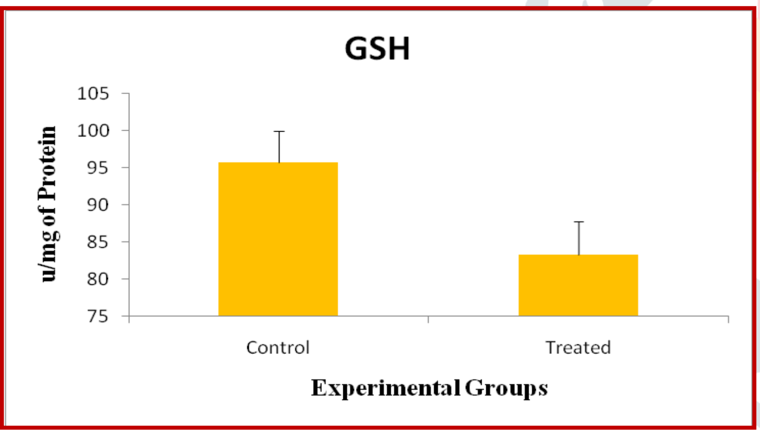


Figure : 19 Biochemical responses GSH activity

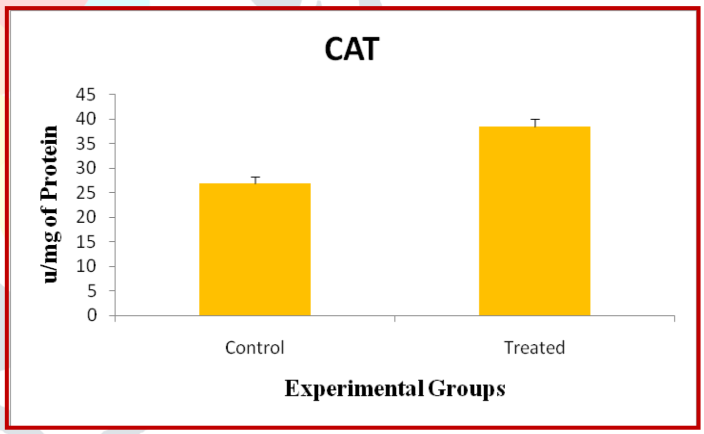


Figure : 20 Biochemical responses CAT activity

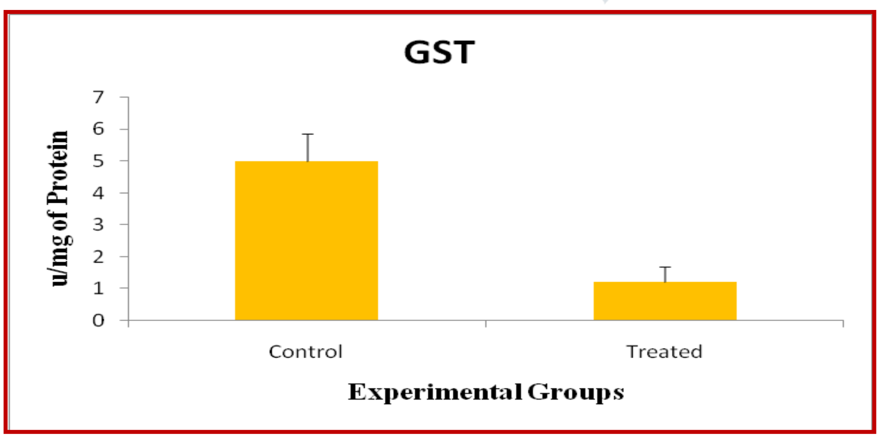


Figure : 21 Biochemical responses GST activity

The effects of fly ash and butter sheet on the biochemical responses of *E. fetida* during vermicomposting are shown in Figs.17, 18, 19, 20 and 21. CAT, GPx and SOD activities were increased and GSH, GST activities were decreased after 60 days. The data clearly reveals that the increased activity of antioxidant enzymes such as SOD, GPx and CAT due to the mixture containing fly ash. Saint-Denis et al. (1998) reported that superoxide dismutase catalyzes dismutation of superoxide anion into O_2 and H_2O_2,while catalase protects the cells by eliminating H_2O_2. CAT and SOD activities were reduced after 60 days treatment. This may be due to the removal of high reactive superoxide of inactivation of SOD by singlet oxygen, hydrogen peroxide and peroxyl radicals (Hodgson and Fridovich 1975; Company *et al.*, 2004; Sandrini *et al.*, 2008). Ribera *et al.,* (2001) reported CAT was used as a biomarker for the study of biochemical responses in earthworm (*E. fetida*) exposed to carbaryl. The reason for the reduction of CAT activity might be high cellular stress or the presence of high levels of ROS. My hypothesis is that the higher concentrations of fly ash and butter sheet mixed treatment might have caused severe stress and must have damaged the cell and DNA of the earthworms. In the present investigation, variation in antioxidant enzyme activities was similar to earlier reports were antioxidant enzyme activities decreased in response to a higher level of mixture of fly ash. Similarly, Honda *et al.*, (2000) concluded that the decrease of antioxidant enzymes may correspond to increased DNA damage.

Conclusion

The present study suggests that through Vermitechnology fly ash can be efficiently recycled into manure by *Eisenia fetida* besides it enhances the nutrient availability of fly ash. Among the various treatments under study 1:3:1 (FA+CD+FW) appeared to be the most efficient. This may be probably as a result of the existence of the maximum microbial population due to sufficient availability of organic material.

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