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# Vermicomposting of herbal pharmaceutical industry waste: Earthworm growth, plant-available nutrient and microbial quality of end materials

Deepika Singh, Surindra Suthar *

*School of Environment & Natural Resources, Doon University, Dehradun 248001, India*

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ABSTRACT

Efforts were made to decompose herbal pharmaceutical industrial waste (HPIW) spiked with cow dung (CD) using *Eisenia fetida*. A total of five vermibeds: $T_1$ – HPIW (0% + CD 100%, control), $T_2$ – HPIW (25%), $T_3$ – HPIW (50%), $T_4$ – HPIW (75%) and $T_5$ – HPIW (100%) were used for vermicomposting. The changes in biology and chemistry of vermibeds were measured after ten days interval. *E. fetida* showed high growth and cocoon production rate in all vermibeds. The vermicomposted material contained great population of fungi 6.0–40.6 (CFU $\times 10^5$ $g^{-1}$), bacteria 220–1276.0 (CFU $\times 10^8$ $g^{-1}$) and actinomycetes 410.0–2962.0 (CFU $\times 10^5$ $g^{-1}$) than initial material. Vermicomposted material was rich in plant-available forms of nutrients ($N\text{-}NO_3^-$, $PO_4^{3-}$, available K and $SO_4^{-2}$). Results suggested that noxious industrial waste can be converted into valuable product for sustainable soil fertility programme.



## 1. Introduction

The industrial development has been included as important venture in modern human civilization and economic reformations of the society. The industrial units produce a huge quantity of both hazardous and non-hazardous solid/liquid wastes, and safe management of such waste has become most challenging issue for the society. The majority of the wastes generated from industrial units are disposed in environmentally unsafe manner which leads to unhealthy urban environment. The herbal pharmaceutical and cosmetic industry in India is probably the oldest medical care system in the world. The use of herbs to cure general health disorders and complex diseases has been mentioned in the Vedas, an ancient religious work of the Hindus. The ancient herbal healing methods of Ayurveda and Unani deal with the use of herbs and natural products to tackle health conditions (Anon, 2011). In recent years the growing concern over side effects of synthetic pharmaceutical and personal care products has resulted in more demand of herbal products in the market. The total market of Ayurvedic products in India is about one billion dollar (US). An estimation suggests about 400,000–500,000 MT consumption per year of key raw material in domestic market for herbal product manufacturing (SME Times, 2010). The herbal-based industrial units produce a huge quantity of spent wastes during pre-processing, oil extraction and distillation processes. In majority of cases there is no direct utilization of such wastes from industrial units and therefore it has become a key issue for local pollution monitoring agencies. The unhealthy open dumping of such waste leads to emission of green house gases, nutrient leaching to surrounding ecosystem and biological contamination of the local bioresources.

Recycling, reuse and resource recovery has been considered as one of the best options for sustainable solid waste management programme. Recovery of nutrients and energy from industrials solid/liquid wastes with low-inputs is now recognized potentially in several sectors of industrial production. Industrial wastes sometimes contain a huge quantity of soil nutrients that could be utilized effectively for crop productions. However, composting is considered as one of the safe option for nutrient recovery from wastes and to reduce waste volume. During composting process detritus community (fungi, actinomycetes, ptotozoa, nematodes, annelids, arthropods etc.) breakdown the complex organic substances into forms which are more available to plants. Vermicomposting, which involves the action of both microbes and earthworm, has been recommended as potential tool to manage organic waste fractions. Vermicomposting is emerging as a most appropriate alternative to aerobic composting (Kale, 1998; Sinha et al., 2010; Hait and Tare, 2011). Vermicomposting has several advantages over conventional thermophilic composting systems in terms of process time, nutrients recovery, microbial richness and phytotoxicity. The quality of end material in vermicomposting process is relatively better than composting in terms of chemical as well as microbial properties. In general it would appear that composted material from vermicomposting can have "added-value" characteristics, mainly related to conservation of nutrients and

* Corresponding author. Fax: +91 135 2533105.
*E-mail addresses:* sutharitd@gmail.com, sutharss_soilbiology@yahoo.co.in (S. Suthar).

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moisture as well as particle size reduction (Abbasi and Ramasamy, 2001; Gomez-Brandon et al., 2011).

The utilization of epigeic earthworms (*Eisenia fetida*, *Eisenia andrei*, *Perionyx excavates* etc.) in waste management practices is well documented in published scientific literature on wastes from industries: vinasse (Pramanik and Chung, 2011), grape mark (Gomez-Brandon et al., 2011), sago industry (Subramanian et al., 2010), paper mill sludge (Elvira et al., 1998), beverage industry (Singh et al., 2010), distillery (Suthar and Singh, 2008), sugar factory (Khwairakpam and Bhargava, 2009; Suthar, 2010), olive oil mill (Moreno et al., 2000) and aromatic oil extraction unit (Deka et al., 2011a,b) etc. The herbal pharmaceutical industry solid waste/sludge may be a potential candidate for vermicomposting operation. No comprehensive report on vermicomposting trial of such waste is yet available in literature. Recently, Deka and his associates studied the vermicomposting of spent waste from units of aromatic oil yielding citronella plant (*Cymbopogon winterianus* Jowitt) using *Perionyx excavatus* (Deka et al., 2011a) and *Eudrilus eugeniae* (Deka et al., 2011b). They claimed that plant waste biomass can be converted into a value-added product employing composting earthworms.

This paper reports the potential of vermicomposting in recycling of plant-available nutrients from waste using epigeic earthworm. Several epigeic earthworms, e.g., *E. fetida*, *P. excavatus*, *P. sansibaricus*, *E. eugeniae*, and *E. andrei* have been identified as detritus feeders and can be used potentially to minimize the anthropogenic wastes from different sources (Suthar, 2007, 2008; Moreno et al., 2000). But *E. fetida* was, and still remains, the favoured earthworm species for laboratory trail experiments on vermicomposting due to its wide tolerance of environmental variables (pH, moisture content, temperature, etc.). Therefore, for this study *E. fetida* was used as vermicomposting agent. The growth and reproduction performance of inoculated earthworms and microbial richness of and material was also explored during this study.

## 2. Methods

### 2.1. Industrial waste, cow dung and E. fetida collection

Originally *E. fetida* used for vermicomposting operation was procured from vermiculture unit, Indian Veterinary Research Institute (IVRI), Izatnagar (Barely), India. The herbal pharmaceutical industrial waste (HPIW) was collected from material processing unit of The Himalaya Drug, Dehradun. Industrial waste composed of unused part of herbs, spent waste from processing and oil extraction unit. The waste was partially shade dried in lab and then stored in dry plastic containers for further vermicomposting operation.

The HPIW was mixed with cow dung (CD) to produce different dilutions of the wastes for further vermicomposting trial. CD was procured from a local cowshed, Mothrawala, Dehradun. The cow dung was partially dried in shade for one week and stored for further experimentations. The chemical characteristic of fresh HPIW and CD is described in Table 1.

**Table 1**
Chemical characteristics ($g\,kg^{-1}$) of industrial waste and cow dung used for experimentation (mean ± SD, $n = 3$).

| Parameters | Cow dung | Herbal pharmaceutical industry waste |
|---|---|---|
| pH | 9.02 ± 0.01 | 8.08 ± 0.22 |
| EC | 0.877 ± 0.02 | 0.264 ± 0.20 |
| Total organic carbon | 743.3 ± 34.4 | 641.26 ± 12.2 |
| $N{-}NO_3^-$ | 29.3 ± 0.90 | 20.42 ± 0.38 |
| Phosphate | 580.0 ± 2.0 | 678.0 ± 2.65 |
| Sulphate | 12.96 ± 0.07 | 14.44 ± 0.22 |
| Available K | 111.50 ± 5.64 | 37.43 ± 1.48 |

### 2.2. Vermibed preparation and observations

A total of five waste mixtures were prepared using different combinations (dry weight proportions) of HPIW and CD (including one treatment without HPIW which acts as experimental control): $T_1$ – 100% CD, $T_2$ – HPIW 25% + CD 75%, $T_3$ – HPIW 50% + CD 50%, $T_4$ – HPIW 75% + CD 25% and $T_5$ – HPIW 100%. For vermicomposting trial, 300 g waste mixture (dry weight basis) was filled in plastic circular containers of 2 L capacity (one for each mixture). The waste mixtures were moistened with distilled water to maintain appropriate moisture level for initial decomposition of waste mixtures. Vermibeds were kept for one week for initiation of microbial degradation and softening of waste mixture. After one week, twenty earthworms having individual weight ~476–552 mg were introduced in each experimental pot container. The experimental beddings were kept in triplicate for each waste mixture. The moisture content was maintained at 55–65% throughout the study period by periodic sprinkling of adequate quantity of water. The containers were placed in a humid and dark place at room temperature (27–28 °C).

About 10 g bedding material was drawn at 0, 10, 20, 20, 40, 50 and 60 days from each experimental container after ten day interval and analyzed for total organic carbon (TOC), Nitrate ($N\text{-}NO_3^-$), phosphate (as $PO_4^{3-}$), sulphate ($SO_4^{2-}$) and available potassium ($K^+$). During first sampling inoculated earthworms were counted for measurement of earthworm mortality (%) in all vermibeds.

The growth and fecundity of inoculated earthworms was also monitored in each experimental container. To measure the changes in live weight of earthworms and newly produced cocoons, the waste mixture was examined after each 10 days interval for 60 days. For that earthworms were separated from the parental waste mixture by hand sorting method, after which worms were washed in tap water to remove adhering material from their body and subsequently weighed, without voiding their gut, and then returned to the original test container. The cocoons produced during the interval were also separated and counted through hand sorting method. Then separated cocoons were incubated in a separate culture container having the same material in which their parents were cultured. Following information about earthworm growth and fecundity was recorded during this study: changes in earthworm biomass, maximum individual weight, maximum growth rate, reproduction rate (cocoon/worm), cocoon production frequency (cocoons/week), initial mortality (%) and biomass gain per unit weight of the feed mixture.

### 2.3. Chemical analysis

Total organic carbon (TOC) was measured after igniting the sample in a Muffle furnace at 550 °C for 60 min by the method of Nelson and Sommers (1996). Extractable phosphorous was measured using the method described by Olsen et al. (1954). Sulphate ($SO_4^{3-}$) was measured by extracting sample with $BaCl_2$. Available potassium ($K^+$) was determined after extracting the sample using ammonium acetate (Simard, 1993). Nitrate ($NO_3^-$) was measured spectrophotomatrically after extracting samples using 0.01 M $CuSO_4$ solution (Jackson, 1975).

### 2.4. Microbial analysis of vermicomposted materials

The vermicomposted material from different experimental waste mixtures were analysed for microbial populations at the end. For this one gram material from each sample was transferred to autoclaved test tubes containing sterilized distilled water, mixed thoroughly using horizontal shaker for 30 min. After that mixture was diluted using serial dilution methods and 1-mL aliquots was plated in autoclaved Petri plates as standard method described in

APHA–AWWA–WPCF (1994). All cultures were kept in triplicate. The pour plate method was used for the enumeration of bacteria, fungi and actinomycetes nutrient agar media, Rose Bengal agar and Kenknight's media, respectively. Pored plates were then incubated for 24, 72 h and one week, respectively to measure the CFU (colony forming unit) of bacteria, fungi and actinomycetes. Microbial counting was measured using the method:

$$\text{Number of CFU g}^{1}\text{ sample} = \frac{(\text{mean plate count})(\text{dilution factor})}{\text{sample weight}}$$

where: CFU = colony forming unit.

### 2.5. Statistical analysis

One-way ANOVA was used to analyze the differences between treatments. A Tukey's *t*-test was also performed to identify the homogeneous type of the data sets. SPSS® statistical package (Window Version 13.0) was used for data analysis. All statements reported in this study are at the $p < 0.05$ levels.

## 3. Results and discussion

### 3.1. Growth, cocoon production and earthworm population in different vermibeds

*E. fetida* showed significant growth and reproduction performance in HPIW mixture during vermicomposting. The changes in individual biomass during vermicomposting process in different waste mixtures are described in Fig. 1.

There was rapid increase in individual biomass of inoculated earthworms in all vermibeds during initial 10 days of vermicomposting except in $T_3$ thereafter; a marked decline was recorded up to the last observation in (Fig. 1) all experimental trials. *E. fetida* showed the peak individual biomass as: 773.77 ± 70.37 mg in $T_1$, 815.17 ± 69.7 mg in $T_2$, 676.55 ± 15.7 mg in $T_3$, 647.64 ± 26.9 mg in $T_4$ and 764.76 ± 34.3 mg in $T_5$. The difference among different experimental waste mixtures for maximum weight achieved was not statistically significant (ANOVA/Tukeys *t*-test; $P = 0.186$). The individual live weight at the end of the experiment was in the ranges of 410.49 ± 13.5 ($T_4$)–604.9 ± 126.7 mg ($T_2$). It is clear that there was weight loss in earthworms during later observation of vermibeds. The loss in earthworm weight is also reported by few other scientists (Neuhauser et al., 1988; Singh et al., 2010). They have attributed the weight loss to food shortage in vermibeds. Probably the conversion of most of the substrate material into vermicompost could not further support the growth in earthworms. The maximum growth rate in *E. fetida* varied among all experimental vermibeds but difference was not statistically significant (ANOVA/Tukeys *t*-test; $P = 0.204$). The maximum growth rate (mg wt. worm$^{-1}$ day$^{-1}$) was 28.93 ± 7.50 in $T_5$ followed by 27.12 ± 8.64 ($T_2$), 22.17 ± 7.35 ($T_1$), 15.73 ± 4.51 ($T_4$) and 7.48 ± 1.20 ($T_3$) (Table 2).

Results of high earthworm biomass gain in pure industrial waste (without bulky agent) bedding (i.e. $T_5$) were unexpected. It is hypothesized that some active ingredients (flavonoids, steroids, saponins, alkaloids etc.) in herbal pharmaceutical waste might be responsible for such growth rate in worms, although further detail study is required to support the hypothesis. Moreover, unusual high feeding rate under stress conditions may lead to weight gain in earthworms and overall better growth patterns in vermibeds with high industrial waste proportion in feed supports this hypothesis. The biomass gained per unit weight of waste is important indicator of suitability of substrate for vermiculture. The biomass gained per unit weight of waste was recorded highest in $T_2$ (2.02 ± 0.73) followed by $T_1$ (1.81 ± 0.28), $T_3$ (1.44 ± 0.33), $T_4$ (1.37 ± 0.78) and $T_5$ (1.18 ± 0.20) (Table 2). The observed difference among vermibeds for weight gained per unit waste could be due to difference in feeding rate and/or growth rate in earthworms. Garg and Gupta (2011) reported biomass gain per unit waste by *E. fetida*, cultured on industrial waste spiked with cow dung, in the ranges of 14.6–17.84. They concluded that industrial waste content affects the biomass gain in earthworms during vermicomposting of industrial wastes.

The reproduction rate (cocoons day$^{-1}$) showed a significant difference among different experimental vermibeds (ANOVA: $F = 26.385$, $p < 0.001$). The maximum rate of cocoon production (cocoons/day) was 5.11 ($T_4$) followed by 3.98 ($T_3$), 3.90 ($T_5$), 2.38 ($T_2$) and 1.35 ($T_1$) (Table 3).

The earthworm showed total cocoon numbers in the ranges of 81.0 ± 9.54 ($T_1$)–306.33 ± 14.31 ($T_4$) in different waste mixtures. The cocoon production in experimental waste mixture was in the

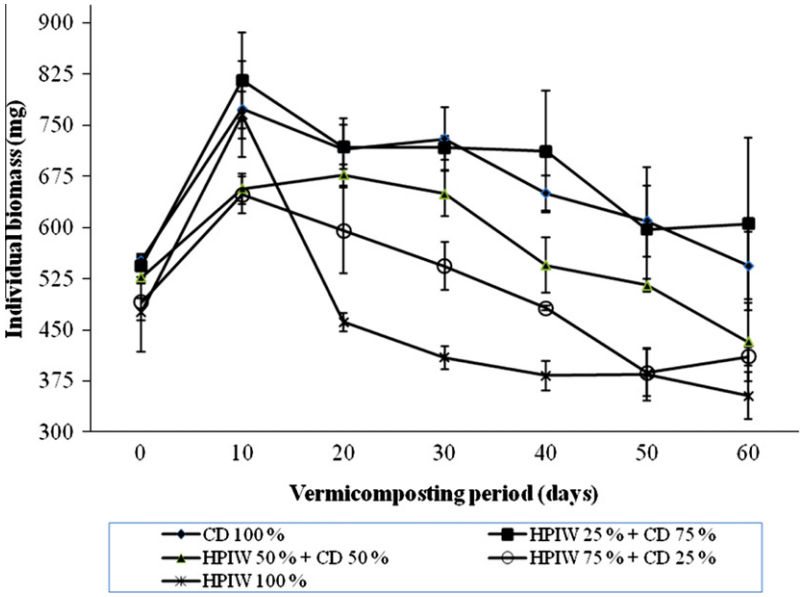


**Fig. 1.** Changes in individual live weight of *E. fetida* during vermicomposting.

**Table 2**
Earthworm productions during vermicomposting process (mean ± SD, $n = 3$).

| Vermibeds | Mean initial biomass of individual earthworm (mg) | Maximum individual biomass achieved (mg) | Net biomass at the end (mg) | Maximum growth rate (mg wt worm$^{-1}$ day$^{-a}$) | Biomass gained per unit waste (mg/g) |
|---|---|---|---|---|---|
| $T_1$ | 552.1 ± 3.97 | 773.77 ± 70.3[a] | 543.92 ± 48.9 | 22.17 ± 7.35[a] | 1.81 ± 0.28 |
| $T_2$ | 544.0 ± 16.7 | 815.17 ± 69.7[a] | 604.9 ± 126.7 | 27.12 ± 8.64[a] | 2.02 ± 0.73 |
| $T_3$ | 527.0 ± 31.3 | 676.55 ± 15.7[a] | 431.9 ± 57.5 | 7.48 ± 1.20[a] | 1.44 ± 0.33 |
| $T_4$ | 490.3 ± 27.2 | 647.64 ± 26.9[a] | 410.49 ± 13.5 | 15.73 ± 4.51[a] | 1.37 ± 0.78 |
| $T_5$ | 475.8 ± 58.7 | 764.76 ± 34.3[a] | 353.16 ± 34.3 | 28.93 ± 7.50[a] | 1.18 ± 0.20 |

[a] Mean value followed by different letters is statistically different (ANOVA; Tukey's *t*-test, $P < 0.05$).

order: $T_4 > T_3 > T_5 > T_2 > T_1$. Hatchling numbers in vermibed varied significant difference among different waste mixtures. The maximum hatchlings were observed in $T_3$ treatment (134.0 ± 4.93) followed by $T_4$ (90.0 ± 5.87), $T_5$ (56.67 ± 1.42), $T_1$ (57.67 ± 5.78) and $T_2$ (41.67 ± 6.77) (Table 3). It is clear that HPIW acts as efficient culture media for earthworm propagations. As observed, the cocoon production was almost better in vermibeds with 50–75% proportion of industrial waste solids suggesting that herbal pharmaceutical waste contains some reproduction stimulating chemical substances. The cocoon production was about 3–3.5 times more in vermibeds containing 75–100% HPIW than treatment with pure CD ($T_1$). Few earlier studies advocated the influence of chemical substances of substrate on reproduction capacity of composting earthworms (Suthar, 2007; Ganesh et al., 2009). The population growth is measured using natality rate (earthworms/parental worm) in organisms. In this study the natality rate in *E. fetida* under different waste mixtures culture was measured. The natality rate in *E. fetida* varied significantly among different waste mixtures (ANOVA: $F = 5.585$, $p = 0.013$). The natality was in the ranges of 2.11 ± 0.55 ($T_1$)–5.62 ± 1.34 ($T_3$). Natality rate in composting worm was in the order: $T_3 > T_4 > T_2 > T_5 > T_1$ (Table 3) Results thus suggested that hatchling success was relatively better in vermibeds with 50–75% industrial waste proportions. At high concentration industrial waste retards the hatchling success of incubated cocoons. This might be due to a variety of factors: temperate, substrate moisture, chemical constituents of culture media etc. Probably the high content of HPIW in substrate cause toxic effects on gametes and further developmental process in cocoons of earthworm. Hence some time high cocoon production rate in earthworms do not help to increase population size in substrate; possibly due to production of non-viable cocoons. Results clearly support the concept that viability of cocoons, laid during vermicomposting process, is equally important in vermicomposting operation.

### 3.2. Microbial population of vermicomposted materials

The microbial populations of vermicomposted waste mixtures were also analyzed. There was statistically significant difference among different vermibeds for microbial population: fungi (ANOVA: $F = 18.19$, $p < 0.001$) bacteria (ANOVA: $F = 7.438$, $p < 0.001$) and actinomycetes (ANOVA: $F = 360.33$, $p < 0.001$) in vermicomposted waste mixture at the end. The highest number of bacteria was in $T_1$ (1276.0 ± 152.4 CFU × $10^8$ g$^{-1}$) while $T_2$ (220 ± 440.0 CFU × $10^8$ g$^{-1}$) showed the lowest bacterial number in ready vermicompost (Fig. 2).

According to the bacterial richness, the vermicomposted waste mixture can be arranged in following order: $T_1 > T_4 > T_5 > T_3 > T_2$. Fungi population was in the ranges of (6.0–40.6 CFU × $10^5$ g$^{-1}$) in different vermibeds. $T_5$ vermibeds showed the maximum fungal population 40.6 ± 16.6 (CFU × $10^5$ g$^{-1}$) while $T_1$ showed the minimum fungal population (6.0 ± 2.65 CFU × $10^5$ g$^{-1}$) (Fig. 2). Similarly, the maximum and minimum population of actinomycetes was in vermicompost obtained from $T_4$ (2962.0 ± 1948 CFU × $10^5$ g$^{-1}$) and $T_5$ (410.6 ± 25.04 CFU × $10^5$ g$^{-1}$). The actinomycetes population in ready vermicompost was in the order: $T_3 > T_1 > T_4 > T_2 > T_5$ (Fig. 2). As described in Fig. 2, the fungi population increased with the increasing proportion of herbal pharmaceutical industrial waste in waste mixture. Fungal population in vermicompost was about 1.2–3.2-fold higher than initial substrates. This suggests the positive impact of earthworm on fungal population in vermicomposting system. The increasing proportion of HPIW caused impact on fungal propagation in vermibeds. Prakash and Karmegum (2010) also reported greater microbial population in vermibed containing partially decomposed leaf of mango. The leaf litter contains a great proportion of cellulose and fungi plays an important role in decomposition of such materials. In general, population of cellulolytic fungi was found to be increased during vermicomposting of different organic wastes (Pramanik et al., 2007). Recently, Pramanik and Chung (2011) reported that increasing proportion of vinasse waste from 25% to 50% or even higher, enhanced the population of both fungi and cellulolytic fungi in waste mixtures used for vermicomposting trials. However, herbal pharmaceutical waste contains several antagonistic chemical substances for microbial growth in wastes. But higher fugal population in vermibeds with increasing proportion of HPIW has suggested no impact of such chemicals on fungal growth.

Bacterial population was higher in CD vermibeds than other treatments. Few earlier scientists have also reported greater bacterial population in vermibeds with high cow dung (CD) proportion (Prakash and Karmegum, 2010; Pramanik et al., 2007). Suthar (2010) suggested that CD contains a number of fungal stains and higher population of other detritus feeders, such as bacteria, protozoa, nematodes, fungi, actinomycetes, which plays an important role in microbial enhancement of vermibeds. John Paul et al. (2011) reported higher bacterial population in municipal solid waste spiked with CD in different ratios. The low bacterial popula-

**Table 3**
Total cocoon production and mortality in *E. fetida* for different vermibeds (mean ± SD, $n = 3$).

| Vermibeds | Total cocoons obtained at the end | Reproduction rate (cocoon/day) | Hatchlings number in vermibeds | Mean mortality rate (%) in earthworms | Natality rate (earthworm/parental worm) |
|---|---|---|---|---|---|
| $T_1$ | 81.0 ± 9.54[a] | 1.35 ± 0.16[a] | 57. 67 ± 5.78[a] | 10.0 ± 0.23[a] | 2.11 ± 0.55[a] |
| $T_2$ | 142.67 ± 8.83[a] | 2.38 ± 0.15[a] | 41.67 ± 6.77[a] | 36.7 ± 7.26[b] | 3.32 ± 0.72[ab] |
| $T_3$ | 238.67 ± 23.56[b] | 3.98 ± 0.39[b] | 134.0 ± 4.93[b] | 0.0 ± 0.0[a] | 5.62 ± 1.34[b] |
| $T_4$ | 306.33 ± 14.31[b] | 5.11 ± 0.24[b] | 90.0 ± 5.87[ab] | 8.33 ± 6.01[a] | 4.87 ± 1.60[ab] |
| $T_5$ | 234.0 ± 23.67[b] | 3.90 ± 0.39[b] | 59.67 ± 1.42[a] | 6.67 ± 3.33[a] | 3.21 ± 0.32[ab] |

Mean value followed by different letters is statistically different (ANOVA; Tukey's *t*-test, $P < 0.05$).

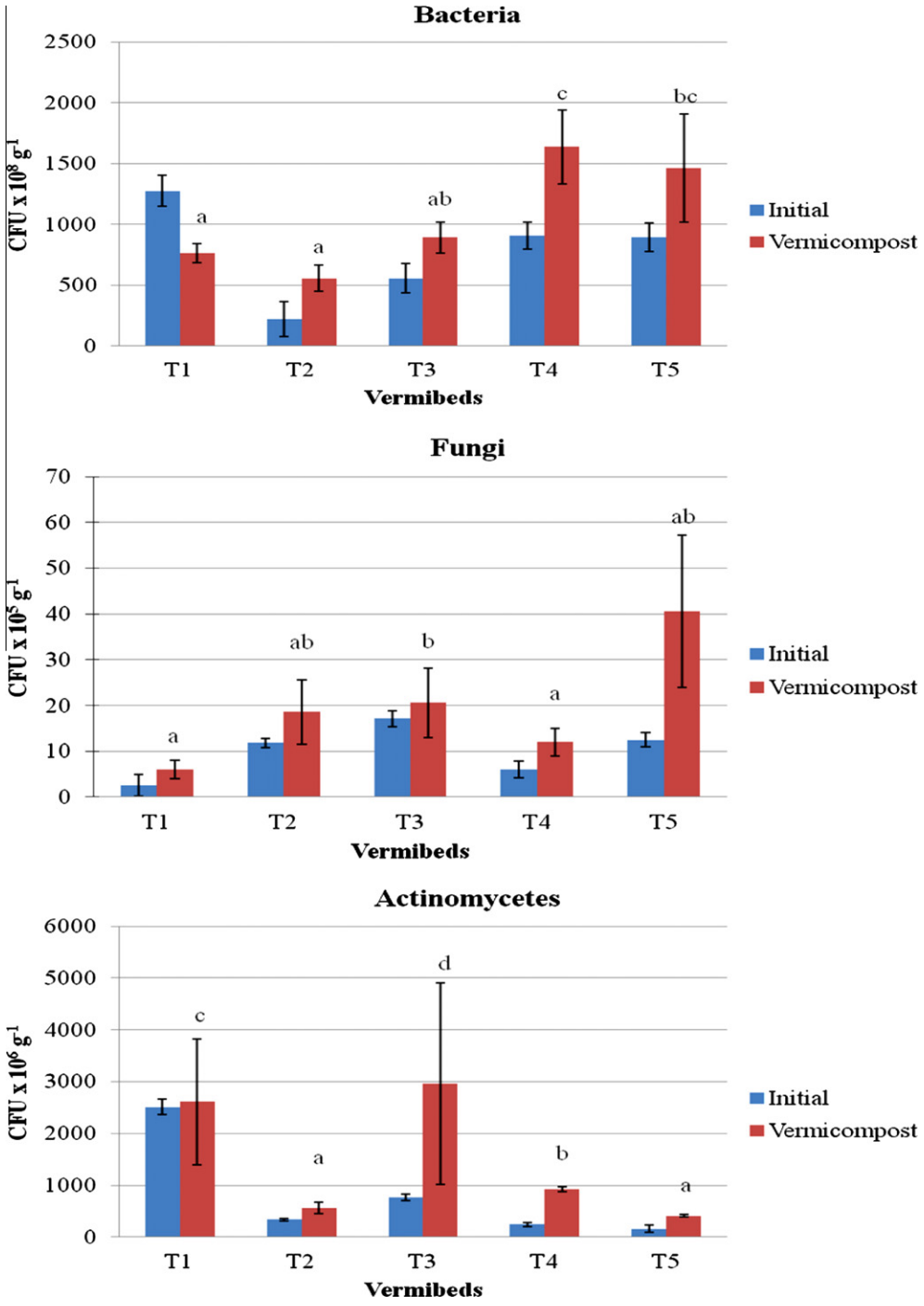


**Fig. 2.** Microbial population of initial and vermicomposted HPIW material.

tion in other vermibeds could be due to chemical characteristics of vermibeds or due to some micro-climatic variability of substrate materials. Bacteria are considered important source of diet of invertebrates especially earthworms. The reduced bacterial numbers in CD attributed to the consumption by inoculated earthworms. Probably bacterial removal rate was higher than population propagation rate in $T_1$. Dominguez (2004) also advocated that detrivorous earthworms can reduce microbial biomass directly by selective feeding on bacteria and fungi, or by accelerating the depletion of resources for microbes. Actinomycetes play an important role in waste mineralization and organic matter decomposition. Actinomycetes are found to be one of the most dominant microorganisms of intestinal microflora of earthworms (Ravasz et al., 1987). All vermicomposted waste mixtures showed the higher population of actinomycetes but no direct relationship between HPIW proportion and actinomycetes numbers in vermibed was observed. Earlier workers have also reported a greater population of actinomycetes in worm-worked materials at the end (Prakash and Karmegum, 2010; John Paul et al., 2011). Kale (1998) suggested that readily available soluble nutrients and processed organic matter in worm-worked material affects the actinomycete count significantly. The antagonistic impact of actinomycetes on litter decomposing fungi is of major concern during vermicomposting of leaf-litter based materials. There was inverse relationship

**Table 4**
Chemical characteristics of initial and vermicomposted industrial waste mixtures (mean ± SD, $n = 3$).

| Treatment | TOC (g kg$^{-1}$) | | | Nitrate (N-$NO_3^-$) | | | Sulphate ($SO_4^{-2}$) | | |
|---|---|---|---|---|---|---|---|---|---|
| | At start | At end | *t*-test (significance) | At start | At end | *t*-test (significance) | At start | At end | *t*-test (significance) |
| $T_1$ | 743.3 (34.35)[a] | 426.10 (35.9) | $p = 0.007$ | 29.30 (0.90) | 32.90 (0.80) | $p = 0.004$ | 12.96 (0.07) | 19.48 (0.09) | $p = 0.007$ |
| $T_2$ | 754.47 (9.34) | 608.8 (10.21) | $p < 0.005$ | 30.62 (0.77) | 26.78 (3.14) | $p = 0.162$ | 11.73 (0.08) | 43.60 (1.00) | $p < 0.005$ |
| $T_3$ | 730.4 (6.39) | 542.2 (27.88) | $p = 0.010$ | 29.82 (0.89) | 28.08 (0.80) | $p = 0.027$ | 12.86 (0.23) | 41.54 (1.75) | $p = 0.001$ |
| $T_4$ | 643.46 (6.07) | 535.6 (15.6) | $p = 0.003$ | 30.52 (2.30) | 26.28 (2.45) | $p = 0.255$ | 12.79 (0.17) | 39.90 (4.46) | $p = 0.008$ |
| $T_5$ | 641.26 (12.20) | 540.9 (7.19) | $p = 0.012$ | 20.42 (0.38) | 29.0 (1.56) | $p = 0.012$ | 14.44 (0.22) | 24.82 (0.23) | $p < 0.005$ |
| | Phosphate ($PO_4^{-3}$) | | | Available K | | | | | |
| $T_1$ | 580.0 (2.0) | 614.30 (5.51) | $p = 0.006$ | 111.5 (5.64) | 144.30 (10.3) | $p = 0.068$ | | | |
| $T_2$ | 610.0 (2.0) | 635.30 (4.16) | $p = 0.013$ | 86.87 (0.98) | 172.30 (18.47) | $p = 0.014$ | | | |
| $T_3$ | 531.76 (2.54) | 562.0 (4.36) | $p = 0.003$ | 53.06 (6.59) | 128.2 (7.93) | $p = 0.012$ | | | |
| $T_4$ | 585.9 (3.43) | 634.3 (5.51) | $p = 0.001$ | 62.93 (6.37) | 108.2 (19.57) | $p = 0.085$ | | | |
| $T_5$ | 678.0 (2.65) | 712.7 (15.37) | $p = 0.069$ | 37.43 (1.48) | 72. 96 (19.40) | $p = 0.076$ | | | |

[a] Values in parenthesis indicates SD.

between fungal and actinomycete population in pure herbal plant waste container especially $T_5$. The low fungal population in this vermibed may be due to high actinomycete biomass, although further detailed studies are required for verifying the science of litter decomposition in earthworm presence. Nevertheless, a recent review by Jayasinghe and Parkinson (2009) also suggested the higher actinomycete population in worm casts which otherwise were antagonistic to surrounding litter decomposer fungi community.

### 3.3. Plant-available nutrients in vermicomposted industrial waste and its agronomic potential

The ready material at the end of the vermicomposting process was analyzed for agronomically important parameters of vermicompost *viz* Nitrate (N-$NO_3^-$), sulphate ($SO_4^{-2}$), phosphate ($PO_4^{-3}$) and available potassium ($K^+$). Nitrate-N is important parameters of compost quality. Nitrate in vermicomposted material was in the ranges of 26.28 ± 2.45–32.92 ± 0.80 g kg$^{-1}$ in different vermibeds. The vermicomposted material showed significant nitrate concentration than initial waste mixtures (*t*-test: $p < 0.001$) except in $T_2$ ($p = 0.162$) and $T_4$ ($p = 0.255$). The maximum increase was in $T_2$ (42.1% more than initial) while $T_3$ showed the minimum increase for $NO_3^-$ contents in final product. Nitrate increase was in the order: $T_2 > T_4 > T_1 > T_5 > T_3$ (Table 4).

The N enrichment process during vermicomposting depends upon the microbial populations and proportion of industrial wastes which contains microbial growth retarding substances (Suthar, 2010). Earthworm enhances the N level of vermibed by adding excreta and other secretions. Also, mucus a polysaccharide is secreted by earthworm to moisten the body surface also important to enrich vermibeds with nitrogen fixers. Earthworm also alters the microclimatic conditions of vermibeds which consequently promotes microbial populations responsible for N enrichment. There was significant increase in the contents of phosphate in all vermibeds than initial levels (Table 4). The level of phosphate in end material was in the ranges of 562.0 ± 4.36 g kg$^{-1}$ ($T_3$)–712.67 ± 15.37 g kg$^{-1}$ ($T_5$) for different waste mixtures. The vermicomposted material showed significantly higher phosphate concentration than initial waste mixtures (*t*-test: $p < 0.001$). The maximum $PO_4^{3-}$ enhancement was in $T_4$ (8.25%) followed by $T_1$ (5.92%), $T_3$ (5.68%), $T_5$ (5.12%) and $T_2$ (4.15%) than initial level. The high available P (plant available form of phosphorus) in vermicompost suggests the agronomic potential of vermicompost as potential plant growth media. Although, available P content in vermicomposted material may reflects the amount of organic forms of phosphorus in waste mixture, but its mineralization rate is determined by the decomposing activities of inoculated earthworms and associated microflora. The phosphorous mineralization is performed partly by earthworm gut phosphatases, and further release of P might be attributed to the microbial activities in deposited casts. Few author suggested the role of P-solubilizing bacteria in phosphorous enhancements in deposited casts of earthworms (Pramanik et al., 2007; Prakash and Karmegum, 2010). The earthworm gut produces considerable amount of alkaline phosphatases, an essential enzyme involved in biogeochemical cycle of P, which facilitate the P mineralization process when waste passes through the worm gut. $PO_4^{3-}$ enhancement showed drastic variations among different experimental waste mixtures. This could be due difference in chemical characteristics of waste feedstock used for earthworm feed. The highest P mineralization in $T_4$ suggests the suitability of waste substrate for earthworm feed and microbial propagation.

The end material showed higher level of available potassium contents than initial levels. The difference between initial and final (vermicomposted) material for available potassium content was statistically significant (*t*-test: $p < 0.001$) except in $T_4$ ($p = 0.085$) and $T_4$ ($p = 0.076$). Available K was the ranges of 72.96 ± 19.40 g kg$^{-1}$ ($T_5$)–172.3 ± 18.47 g kg$^{-1}$ ($T_2$) in end product of vermicomposting process. The available K also showed drastic variations among different vermibeds and the maximum increase was in $T_3$ (145.5% than initial) followed by $T_5$ (98.3%), $T_2$ (93.9%), $T_4$ (74.7%) and $T_1$ (29.9%) (Table 4). The waste mineralization mainly depends upon the earthworm activity and microbial population in waste mixture. In general, when organic waste passes through the gut of worm some fraction of organic minerals is then converted into more available species of nutrients (i.e. exchangeable forms) due to the action of endogenic and/or exogenic enzymes (Suthar, 2010). This result agrees with previous reports that the vermicomposting accelerate the waste mineralization rate by facilitating the microbial communities in decomposing waste sub-systems. The high range of potassium in vermicomposted material further indicates the agronomic importance of end products.

Sulphate content was higher (Table 4) in vermicomposted materials than initial level (*t*-test; $p < 0.001$). $SO_4^{2-}$ content was in the ranges of 19.48 ± 0.09–43.60 ± 1.00 g kg$^{-1}$ in different vermibeds. $T_3$ vermibed showed the maximum $SO_4^{2-}$ increase (271.7% more than initial level) followed by $T_3$ (222.8%), $T_4$ (211.8%), $T_5$ (71.9%) and $T_1$ (50.4%). Data clearly suggested the role of earthworm in sulphur mineralization in vermicomposting process. Earthworm modified the waste physical as well as chemical characteristics which results in enhanced microbial activities in vermicomposting sub-system. In addition to bacteria the fungal also plays an important role in waste mineralization during vermicomposting process. The observed difference among different treatment beddings could be due to different in feed quality and/or microbial populations. The impact of earthworm on sulphur mineralization is not well

discussed by previous authors therefore; a details studies about organic sulphur transformation during vermicomposting process need to be investigated in details.

## 4. Conclusions

This work presents the utilization of HPIW in vermicomposting operation. Results thus clearly suggested that vermicomposting is significantly effective in nutrient transformations in waste mixtures. Also the microbial population was manifold in worm-processed wastes than initial waste mixture. The growth and cocoon production rate of *E. fetida* was better in all HPIW waste mixtures. This study advocated the candidature of HPIW for vermicomposting operation to address the issue of sustainable industrial development.

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