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Evaluation of the Concentration of Phytotoxic Chemicals and Microbial Load of the Vermicompost Prepared from Coffee Processing Waste

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Abstract Coorg, a district in the Indian state of Karnataka, is well known for producing and exporting coffee and is also known as the "Coffee Land of India". Industries that process coffee produce enormous amounts of waste, including coffee husk and pulp. The disposal of this waste is a significant problem. The simplest method of waste disposal is tossing it into landfills, resulting in serious eco-toxicological issues such as the leaching of phytotoxic chemicals such as caffeine, tannins, and polyphenols. Additionally, the microbial load of the plant growth-promoting microorganisms in the contaminated soil gets diminished due to these toxic substances. The current study aimed to determine the feasibility of reducing the phytotoxic chemicals in the soil contaminated with coffee processing waste by generating compost and vermicompost. This study analyzed three treatments of soil: first treatment comprised soil polluted with coffee processing waste; in the second treatment the soil polluted with coffee processing waste was treated with cow dung, weeds and chopped banana leaves, native soil in the ratio 6;2;1;1 and the natural compost was generated. Treatment 3 was prepared by keeping treatment 2 for 15 days for pretreatment and it was inoculated with adult earthworm *Eudrilus eugiensis* and vermicompost was prepared. All treatments were kept for 90 days. The concentration of phytotoxic compounds such as caffeine, chlorogenic acid, and tannins in three treatments was examined, every 15 days over the course of 90 days. Triplicates of the measurements were done and the average values were taken for conclusions. It was found that the concentration of caffeine, chlorogenic acid, and tannins dramatically decreased from 1.25, 0.66, and 0.35 mg/gm to 0.05, 0.0523, and 0.02 mg/gm respectively after vermicomposting. Also, the treatment was found to have a gradual increase in the microbial load of plant growth-promoting microorganisms, including *Pseudomonas*, nitrogen-fixing microorganisms, phosphate-solubilizing microorganisms, starch hydrolyzing microorganisms, pectinolytic and chitinolytic microorganisms. Thus, the research proved that composting and vermicomposting are the best options for

recycling coffee processing waste.

Keywords Coffee Processing Waste, Phytotoxic Chemicals, Microbial Load, Microorganisms, Compost, Vermicompost, Cow Dung, *Eudrilus eugiensis*

1. Introduction

Coffee is an important cash crop and the most traded commodity after petroleum products. Coffee is widely cultivated in Coorg, a district in Karnataka state, India [1]. Thus, it is also known as the coffee land of India as it is well-known for its coffee production and export. It has a planted area of 1, 07,089 hectares and produced 1, 24,950 metric tonnes of coffee in 2020-2021 [2]. After harvesting, the coffee fruit is processed either by wet or dry methods, yielding coffee pulp and coffee husk respectively (Figure 1). The coffee processing waste accounted for 40-50 percent of the weight of the coffee fruit [3]. Because coffee may only be sold after it has been processed, any waste generated should be managed by the region where it is grown. As a result, the entire coffee waste stream is disposed of in landfills [4].

The presence of phytotoxic compounds, such as caffeine, polyphenols, and tannins in coffee processing wastes, limits its use as a value-added product and pollutes the soil [5]. If the phytotoxic compounds of concern can be reduced to subtoxic levels, the organic carbon, nitrogen, phosphorous, and potassium content of coffee pulp and coffee husk makes it be used as a good fertilizer [6]. Our research aimed to reduce the concentration of phytotoxic compounds in coffee processing waste to convert it to high-quality vermicompost.

Organic materials undergo spontaneous biological degradation during composting, mostly in an aerobic atmosphere. Avoiding environmental pollution and the immobilization of nutrients is just one benefit of using organic compost in agriculture, which also serves as a source of organic matter for the soil [7]. By- products of coffee would be treated using biological processes that are oxygen-driven in order to produce fertilizer while also protecting the environment. In this regard it was observed that treating coffee waste by composting lessens the significant harm that would be caused by applying immature compost to the soil and enable complete conservation of the residual energy held in the organic material [8].

The process of aerobic decomposition of organic materials with the help of earthworms is known as vermicomposting. It is an environmentally benign method of recycling biogenic waste that would otherwise pollute the environment [9]. The method is the least expensive and produces high-quality vermicompost. Vermicompost is a peat-like substance with excellent aeration, porosity, drainage, and water-holding capacity [10]. Vermicompost is rich in macro and micronutrients, and trace elements in plant-available forms. It also contains plant growth hormones, enzymes, and helpful microorganisms, according to reports [11]. There are scientific reports of increased concentrations of macro and micronutrients in the vermicompost prepared from coffee processing wastes [12, 13]. Hence the main objective of this research is to analyze the chemical content and the presence of micro-organisms in compost and vermicompost.

a). Coffee husk b). Coffee pulp

Figure 1. Coffee processing waste

2. Review of Literature

Over the course of 90 days, Werako *et al* [14], studied the windrow composting of coffee husk and pulp together with other organic wastes using six different treatments that were accessible. A Randomized full-block design was used to perform six separate treatments. The composting of coffee husk and pulp with that waste and cow dung was found to be the optimum method, according to the compost quality based on the physicochemical and nutritional content of the matured compost.

Mahboub *et al* [15], analyzed the growth factors and nutrient results in *Dracaena*, *Spathiphyllum*, and *Aglonema* and revealed that the application of 25% of the two types of vermicompost significantly affected the height and diameter of the plant as well as the nitrogen, phosphorus, and potassium of the leaves and caused a significant difference with the control. According to observations, adding more vermicompost enhanced the amount of nutrients in the leaves compared to the control. However, a lower yield at greater vermicompost concentrations was observed to be caused by the inhibitory impact of rising EC and pH as well as unfavorable physical characteristics of the growing medium.

Purwanto *et al* [4], created vermicompost from Skin Coffee (SC) modified with Green Waste (GW) and Biochar (B) and assess their impact on the development of the coffee plant. A carbon-rich material called biochar is created by pyrolyzing rice husks at 4500°C together with green waste, such as branch clippings and leaves from Universitas Negeri Jakarta's garden and coffee skins from Cibulao Coffee Farm in Bogor, Indonesia. The variables of plant height and wet weight as well as dry weight of coffee seedlings in the main nursery were significantly affected by the treatment of medium composition with coffee husk waste vermicompost.

Sibomana *et al* [16], carried out a triple experiment on two cabbage (*Brassica oleracea L.*) varieties (*Mukasi and Kidodo*) and on eggplant in order to assess the fertilizer potentials of coffee pulp composts enhanced with (micro) biological accelerators on potato (*Solanum tuberosum L.*) and bean (*Phaseolus vulgaris L.*) crops (*Solanum melongena L.*). The Coffee pulp and bean residue treatment was found to be more affordable than the costly inorganic fertilizers and more replicable by farmers (because of the availability of the product locally) than the employment of efficient microbes. Regarding the expense of procurement (importation), conservation, manipulation, and accessibility to underprivileged rural Burundian farmers, the latter treatment presents difficulties.

Aslam *et al* [17], examined different vermicompost, such as rice straw, wheat straw, and cow dung in varying quantities, nine treatments were employed. The quantity of earthworms, nutrients, and cocoons increased when vermicomposting was done with cow dung and microbial strains, rice straw and microbial strains, and decreased when vermicomposting was done with wheat straw. Total nitrogen concentration of vermicompost was found to be varied from 0.90 to 2.2 percent and was greater than the raw material's 0.23 to 1.09 percent, thus showing how hazardous waste might be converted into beneficial fertilizers. According to the findings of the present study, cow dung was the best source for boosting the availability of both macro and micronutrients.

Thus, to have a virtuous benefit over other techniques, chemical characteristics of the soil has to be realized to determine various method to generate the necessary treatment to reduce the chemical content in order to increase the presence of microbial content in the soil. The sections below describe the study location and the process involved in the various treatment used for the identification of chemical content decreased in the soil contaminated with soil processing waste.

3. Material and Methods

To evaluate the feasible treatment of soil contaminated with coffee processing waste this research collected three differently treated soil with coffee processing waste and created three different specimens. Then the specimen was observed for 90 days at an interval of 15 days. The materials and methods used to predict the results are elaborated in detail in this section.

3.1 Study Location

The experiments in this research were conducted in 7th Hoskote, located at 12˚26̀ 22.82″ N and 75˚52̀ 26.82″ E, Somvarpet Taluk, Coorg, Karnataka, India [18]. With approximately 270 genera of shaded tree varietals, Coorg's coffee agro forestry system is one of the world's richest agroforestry systems. The Coffee Board of India classifies 98.8% of coffee holdings in Coorg as small (less than 10 hectares), accounting for 70% of total production, while 1.2% of holdings as large, accounting for roughly 30% of total coffee production.

3.2. Study Material

Generally, there are two methods for processing coffee cherries which are dry and wet methods. These release solid waste such as coffee husk and coffee pulp. The coffee husk contains some amount of caffeine and tannins, which can make it toxic and slow degradation in nature, resulting in the disposal problem. However, the coffee husk is rich in lignocellulose materials, which makes it an ideal substrate for microbial processes [19]. The disposal of these coffee processing wastes tends to be a tedious disadvantage to the soil where these coffee processing wastes are dumped due to the increased chemical content

which kills the essential micro-organism naturally present in the soil.

Figure 2. Coffee processing waste dumping area in Coorg, 7th Hoskote

Due to the regular dumping of coffee processing waste the waste disposal place in Coorg looks as in Fig 2 which is a regular scene. This image was collected directly by observing the place. In this research, the coffee husk utilized was collected from one private Coffee curing work in, Industrial area, Kushal Nagar. Also, the coffee pulp used was obtained from a pulping unit of Ganesh Estates, Nakoor Sirankala. As cow dung increases the presence of microorganism content in the compost was also used in this research which was collected from Nakoor Sirankala.

3.3. Study Specimens, Earthworms

Figure 3. Eudrilus eugeniae

The earthworm, *Eudrilus eugeniae* shown in Fig 3 belongs to the phylum Annelida, class: Clitellata, and family: Eudrilidae, which is commonly known as the "African Night Crawler". The worm is a great model for cell and molecular biological experiments [20]. In this research earthworm, *Eudrilus eugiensis* was purchased from Ganapathi Nursery, Upputhodu, Somvarpet, and Coorg. Topsoil used for the work has been taken from 7th Hoskote.

3.4. Preparation of the Composting Material and Pre-treatment

3.4.1. Treatment 1: (T1)

Soil polluted with coffee processing waste such as coffee husk and coffee pulp was directly collected from Coorg region. Ratio of coffee processing waste: native soil was taken as 8:2, where in which the concentration of phytotoxic chemicals initially was found to be 1.32mg/gm of caffeine, 0.68 mg/gm of chlorogenic acid and 0.45 mg/gm of tannins.

3.4.2. Treatment 2: (T2)

A mixture of coffee processing waste, cow dung, weeds and chopped banana leaves, and native soil was mixed and the compost was prepared (Natural composting). The ratio of coffee processing waste: (cow dung + weeds + chopped banana leaves): native soil was taken as 6:3:1.

3.4.3. Treatment 3: (T3)

The T2 mixture was kept aside for a period of 15 days for natural decomposition, and then it was inoculated with adult earthworms (1%), and the vermicomposting was prepared for the analysis. In the 6:3:1 ratio the coffee processing waste: (cow dung + weeds + chopped banana leaves): native soil was mixed.

Experiments were performed in brick tanks measuring 1m X 1 m X 0.75 m (length X breadth X depth) with overhead shade and proper drainage facilities. The experiment was performed in natural conditions as it was there in 7th Hoskote, Somawarpet, and situated 891m above sea level where the average temperature was found to be between 20-26°C and humidity of 85-90%.

4. Analysis of the Results

The specimen that was collected from three various treatments was kept for 90 days and at an interval of 15 days it was investigated and the following results were obtained and are discussed in detail in the following section.

4.1 Chemical Analysis

Quantification of chemicals such as caffeine, chlorogenic acid and tannins was done in this research. Quantitative estimation of caffeine was made using the standard protocol with appropriate modifications for soil samples. The chlorogenic acid concentration was measured quantitatively using the Folin-Ciocalteu reagent (Sigma) and the Folin-Denis reagent (Sigma) was used for the quantification of tannins. The presence of the chemicals was quantified and a graph was plotted for three treatments [21].

4.1.1. Caffeine

Soil polluted with the coffee processing waste (T1) was found to have 1.32mg/g of caffeine as in table 1. Even after 90 days there was no significant reduction in the caffeine content (0.07mg/g). In the pre-treated coffee waste, the caffeine content decreased up to 0.88mg/g and after 90 days of natural composting (T2), it became 0.09mg/g. In vermicomposting (T3) significant reduction in the caffeine content was noticed and it was found to be 0.05mg/g.

The plotted graph in Fig.4 shows the presence of caffeine in three treatment samples. It is clearly visible that the 3rd treated sample has the least amount of caffeine. On the 90th day treatment 1, treatment 2, and treatment 3 had 0.07 mg, 1.21 mg and 1.27 mg reduced respectively.

4.1.2. Chlorogenic Acid

Soil polluted with coffee waste (T1) was found to have 0.68mg/g of chlorogenic acids. Keeping the polluted soil as such without any treatment did not show any significant change in the chlorogenic acid content (0.66mg/g) as in table 2. In the pre-treated coffee waste, the chlorogenic acid content decreased up to 0.02mg/g and finally after 90 days of composting (T2), the chlorogenic acid content became 0.065 mg/g and in vermicomposting (T3), it was 0.0523mg/g. A significant reduction of chlorogenic acid content took place both in composting (T2) and vermin composting (T3).

Table 1. Presence of caffeine in the treatments

Days	T1(mg/g)	T2(mg/g)	T3(mg/g)
1st	1.32	1.32	1.32
15th	1.3	0.93	0.92
30th	1.29	0.61	0.55
45th	1.29	0.49	0.43
60th	1.28	0.4	0.22
75th	1.26	0.14	0.1
90th	1.25	0.11	0.05

Table 2. Presence of chlorogenic acid in the treatments

Days	T1(mg/g)	T2(mg/g)	T3(mg/g)
1st	0.68	0.72	0.72
15th	0.678	0.238	0.239
30th	0.676	0.173	0.161
45th	0.675	0.157	0.144
60th	0.669	0.09	0.064
75th	0.665	0.075	0.055
90th	0.66	0.065	0.0523

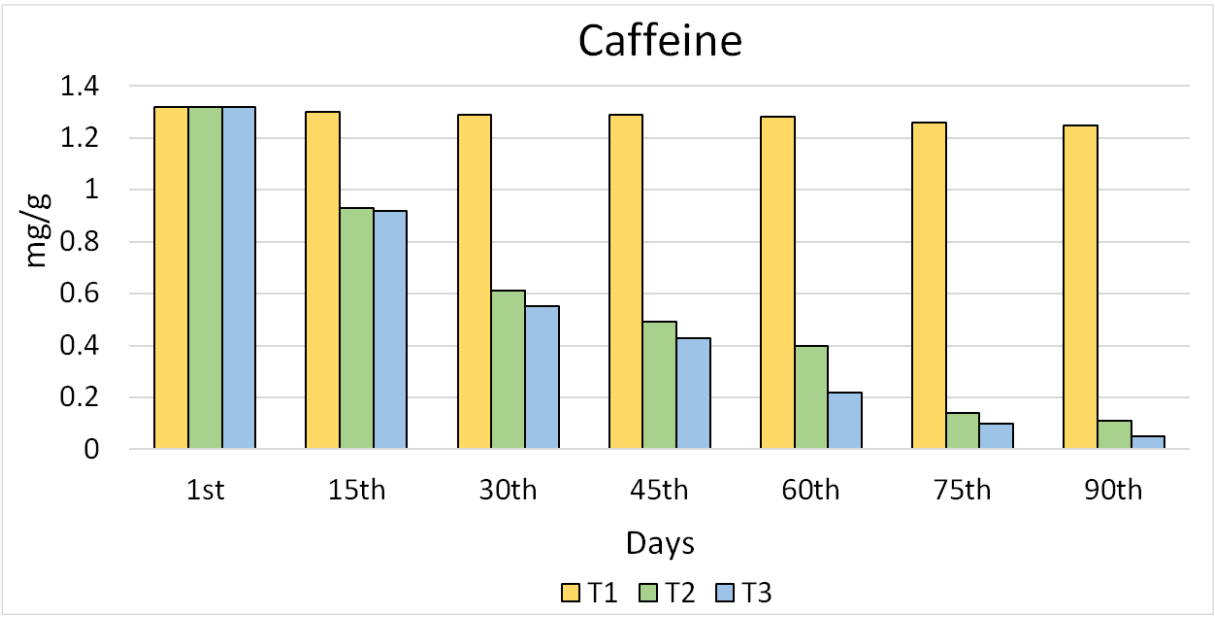

Figure 4. Presence of caffeine in the treatments

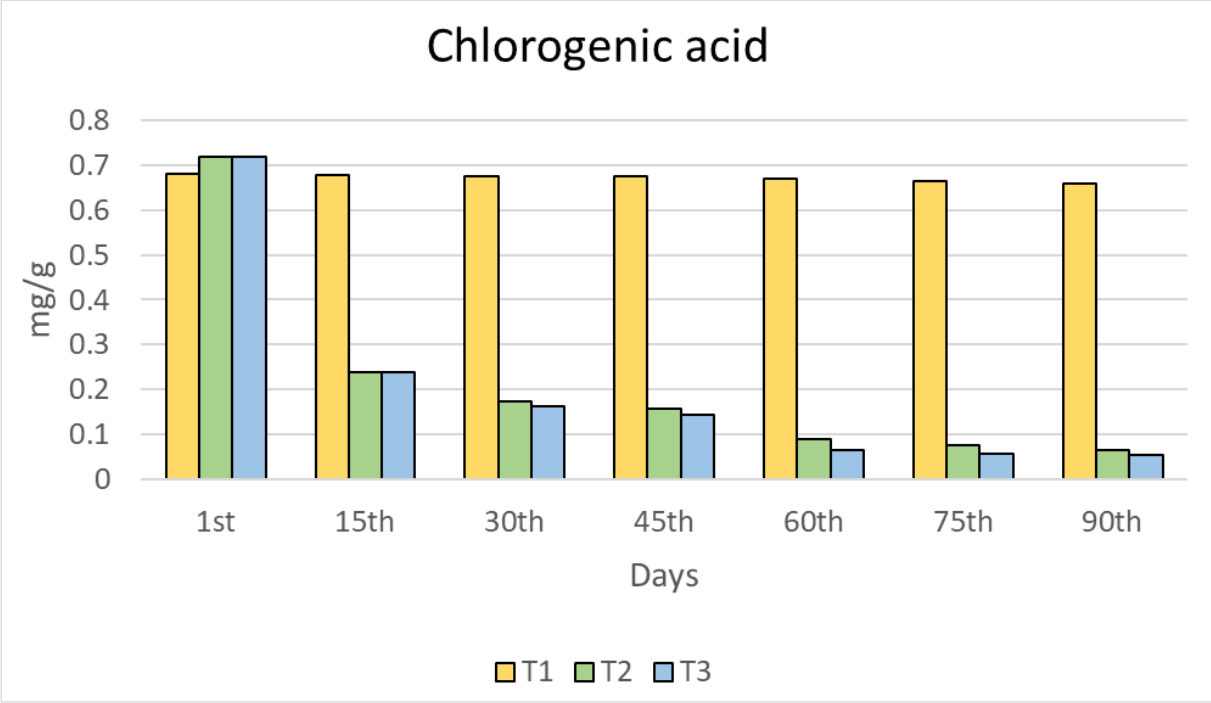

Figure 5. Presence of chlorogenic acid in the treatments

The presence of chlorogenic acid in three treatment samples is depicted in the plotted graph in fig.5. The 3rd treated sample has the least quantity of caffeine, as can be seen. On the 90th day, the presence of chlorogenic acid in treatment 1, treatment 2, and treatment 3 was reduced by 0.02 mg, 0.655 mg, and 0.667 mg, respectively.

4.1.3. Tannins

Soil polluted with coffee waste (T1) was found to contain 0.45mg/g of tannins. Even after 90 days the reduction in tannin content was not significant (0.10mg/g) as in table 3. On the other hand, the pre-treatment of coffee waste reduced the tannin content to 0.12mg/g, and after 90 days of composting it became 0.03mg/g and for vermicomposting (T3) it became 0.02mg/g.

Table 3. Presence of tannins in the treatments

Days	T1(mg/g)	T2(mg/g)	T3(mg/g)
1st	0.45	0.56	0.56
15th	0.43	0.14	0.13
30th	0.42	0.09	0.08
45th	0.42	0.07	0.06
60th	0.4	0.05	0.04
75th	0.38	0.04	0.03
90th	0.35	0.03	0.02

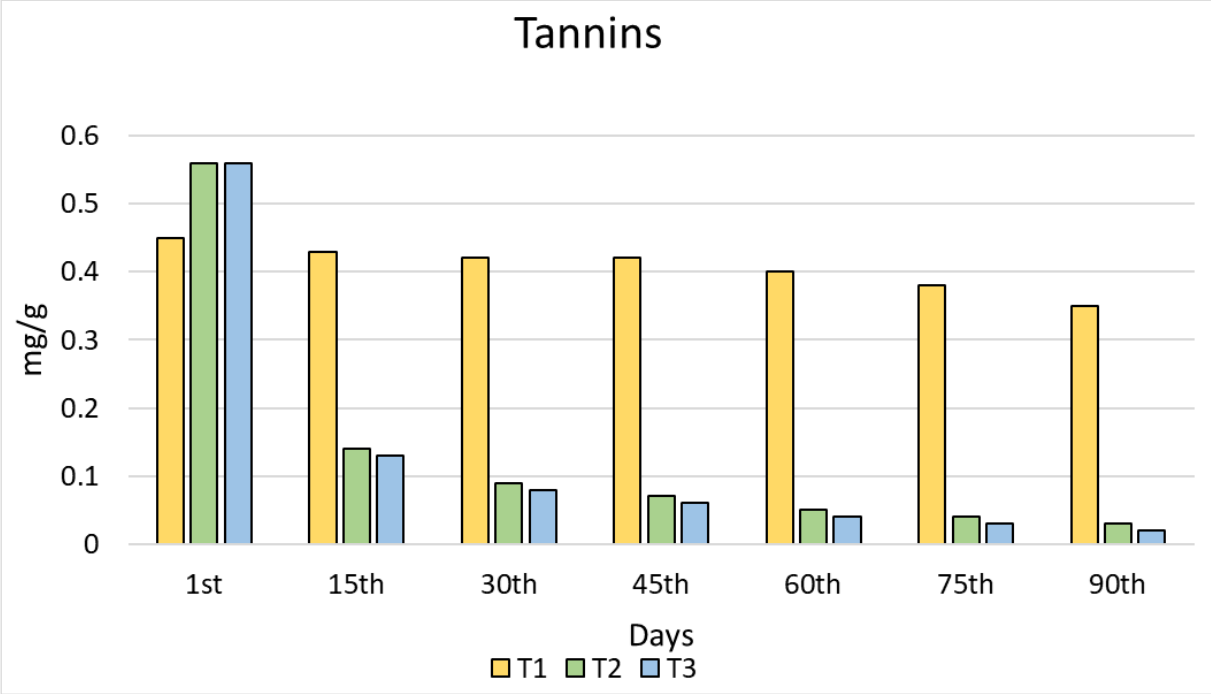

Figure 6. Presence of tannins in the treatments

In the plotted graph in fig. 6, the presence of tannins in three treatment samples is indicated. As can be observed, the third treated sample contains the least amount of tannins. In treatment 1, treatment 2, and treatment 3 the amount of tannins was reduced by 0.07 mg, 1.21 mg, and 1.27 mg, respectively, on the 90th day.

4.2. Microbial Analysis

Samples of soil were taken and three different treatments were prepared and the presence of micro-organisms and their growth was investigated at 15 days intervals of time. For investigation 0.1g of soil was serially diluted in 90mL ringer solution up to 10^{-6} dilutions and 1mL aliquot was pour-plated in selective media. Nutrient agar for bacteria, Cooke rose Bengal for selective cultivation and isolation of fungi, Actinomycetes isolation agar for the isolation and cultivation of Actinomycetes, Malt extract agar for the isolation and cultivation of yeast, Jensen's media for the isolation and cultivation of nitrogen-fixing bacteria, Pikovskaya's media for the detection of phosphate solubilising soil microorganism, Starch agar media for starch hydrolyzing microorganism, King's media for the isolation and cultivation of *Pseudomonas sp*. was used for culturing the specific microorganism. The plates were incubated at 25±10°C. All the tests were done in triplicates. Special microorganisms like mineralizers and polymer degraders were enumerated using the specific media following standard microbiological methods [22]. After the incubation period the microbial colonies were counted as Colony-Forming Units per gram (CFU/g) of the soil sample. The colony characteristics were observed and single colonies were isolated and sub cultured on the respective media and the representative single colonies were identified using Bergey's Manual of Determinative Bacteriology. Identification of yeast and fungi was done as per the available standard manual.

4.2.1. Bacteria

Table 4. Presence of Bacteria in the treatments

	T1(CFU×10^6/g)	T2(CFU×10^6/g)	T3(CFU×10^6/g)
1st	50	101.78	102.01
15th	52.67	105.01	108.69
30th	55.63	107.91	110.43
45th	58.19	112.72	127.98
60th	62.23	115.68	129.83
75th	65.45	118.26	130.75
90th	67.01	121.42	132.64

The number of bacteria in soil polluted with coffee waste (T1) was found to be 50 CFU × 10^6/g as in table 4. The bacteria content of the polluted soil did not vary significantly (67.01 CFU × 10^6/g) when it was left untreated. The bacteria content of pre-treated coffee waste raised to 121.42 CFU × 10^6/g after 90 days of composting (T2) and 132.64CFU × 10^6/g after 90 days of vermicomposting (T3) as shown in fig 7. Both composting (T2) and (T3) vermi composting had a considerable increase in bacteria content.

4.2.2. Fungus

The fungus microbes in coffee waste-polluted soil (T1) were discovered to be 15.08 CFU × 10^6/g as in table 5. When the polluted soil was left untreated, the fungus content did not change considerably (67.01 CFU × 10^6/g). After 90 days of composting (T2), the fungus content of pre-treated coffee waste increased from 25.07 to 34.25 CFU × 10^6/g, and after 90 days of vermicomposting, it increased from 25.08 to 52.03 CFU × 10^6/g (T3) shown in fig 8. The fungus level of both composting (T2) and vermicomposting (T3) increased significantly.

Table 5. Presence of fungus in the treatments

	T1(CFU×10^6/g)	T2(CFU×10^6/g)	T3(CFU×10^6/g)
1st	15.08	25.07	25.08
15th	18.83	30.49	30.56
30th	20.55	31.08	34.34
45th	21.16	31.93	38.18
60th	22.76	32.24	45.78
75th	23.98	33.67	48.33
90th	24.5	34.25	52.03

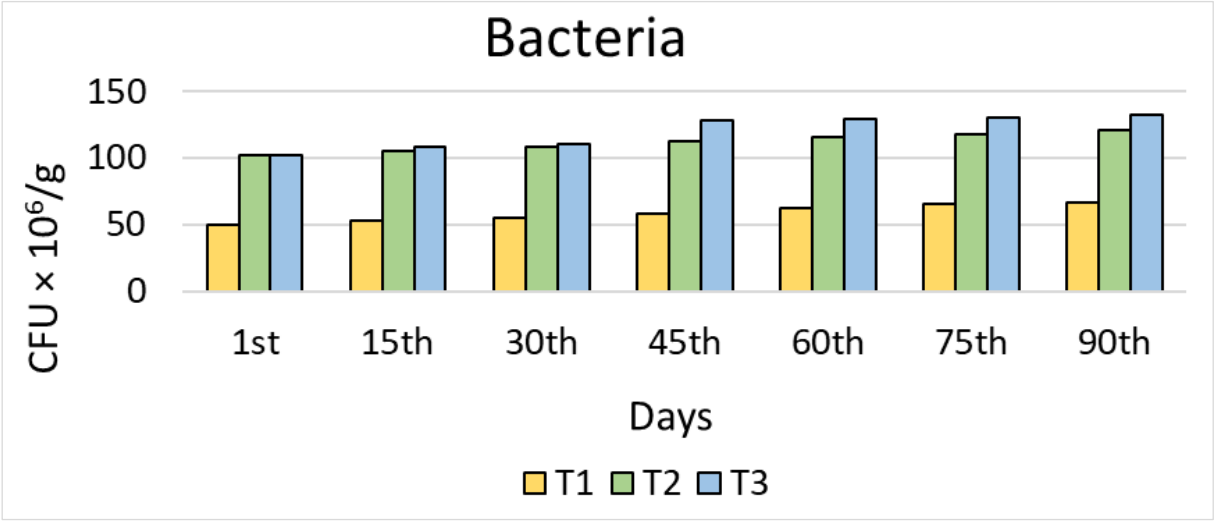

Figure 7. Presence of Bacteria in the treatments

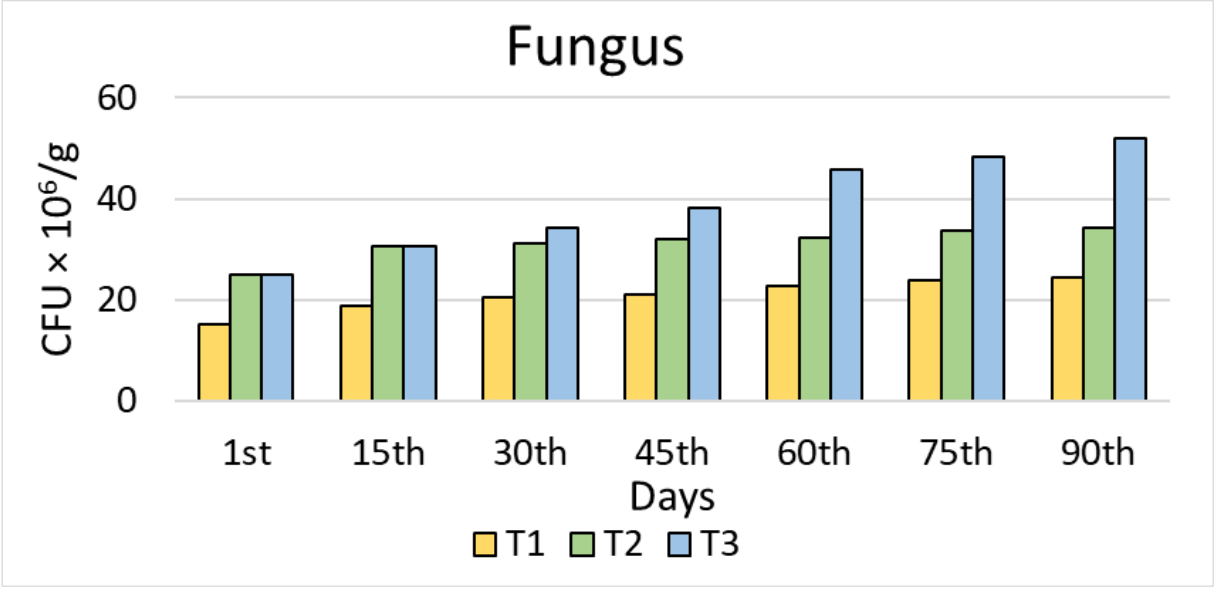

Figure 8. Presence of fungus in the treatments

4.2.3. Yeast

The amount of yeast found in coffee waste-polluted soil (T1) was 22.05 CFU × 10^6/g. The yeast concentration did not alter much when the contaminated soil was left untreated (33.31CFU × 10^6/g) as in 6. The yeast content of pre-treated coffee waste grew from 35.01 to 110.33 CFU × 10^6/g after 90 days of composting (T2), and from 36.07 to 131.79 CFU × 10^6/g after 90 days of vermicomposting (T3) as shown in Fig. 9. Both composting (T2) and vermicomposting (T3) yeast levels dramatically increased.

Table 6. Presence of yeast in the treatments

Days	T1(CFU×10^6/g)	T2(CFU×10^6/g)	T3(CFU×10^6/g)
1st	22.05	35.01	36.07
15th	25.46	43.06	43.05
30th	28.93	101.39	111.39
45th	29.97	106.49	120.44
60th	31.01	108.36	126.56
75th	32.71	109.43	128.8
90th	33.31	110.33	131.79

4.2.4. Actinomycetes

In coffee waste-polluted soil (T1), the number of actinomycetes discovered was 32.76 CFU × 10^6/g as in Table 7. When the polluted soil was left untreated (44.38 CFU × 10^6/g), the concentration of actinomycetes did not change appreciably. After 90 days of composting (T2), the yeast content of pre-treated coffee waste increased from 52.75 to 113.49 CFU × 10^6/g, and after 90 days of vermicomposting, it increased from 52.76 to 140.19 CFU × 10^6/g (T3) as shown in Fig. 10. The levels of composting (T2) and vermicomposting (T3) actinomycetes also increase rapidly.

Table 7. Presence of actinomycetes in the treatments

Days	T1(CFU×10^6/g)	T2(CFU×10^6/g)	T3(CFU×10^6/g)
1st	32.76	52.75	52.76
15th	34.86	60.45	60.48
30th	35.01	90.76	116.48
45th	37.43	93.19	125.19
60th	40.56	98.15	133.35
75th	41.04	100.13	137.76
90th	44.38	113.49	140.19

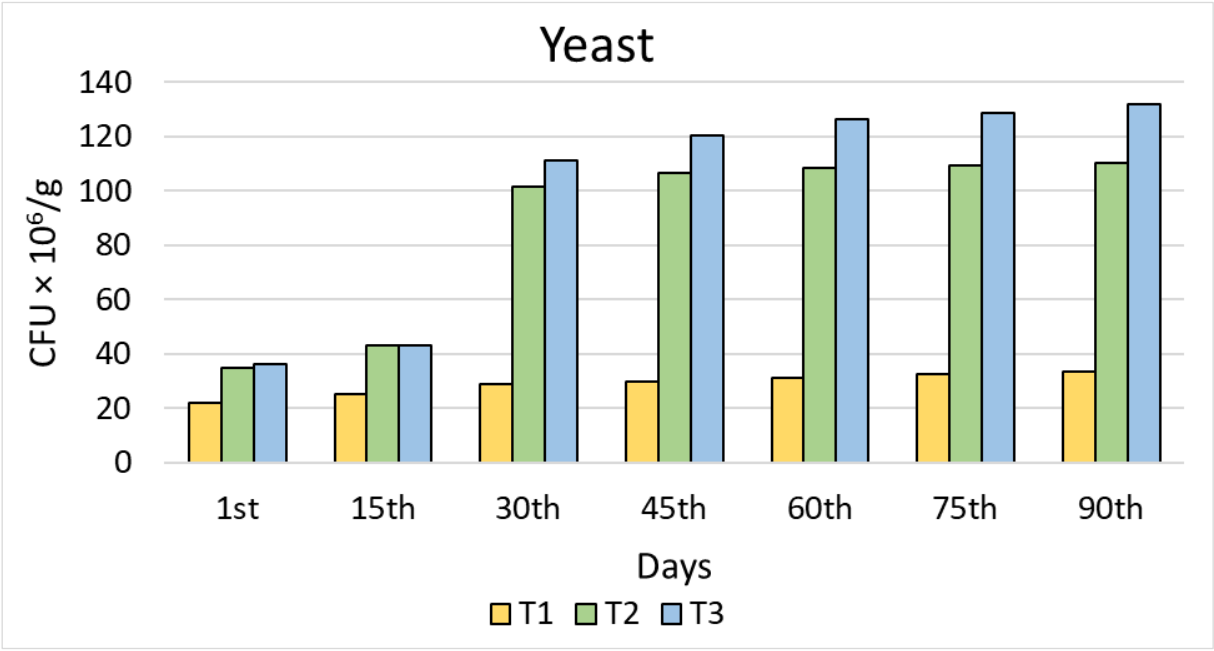

Figure 9. Presence of yeast in the treatments

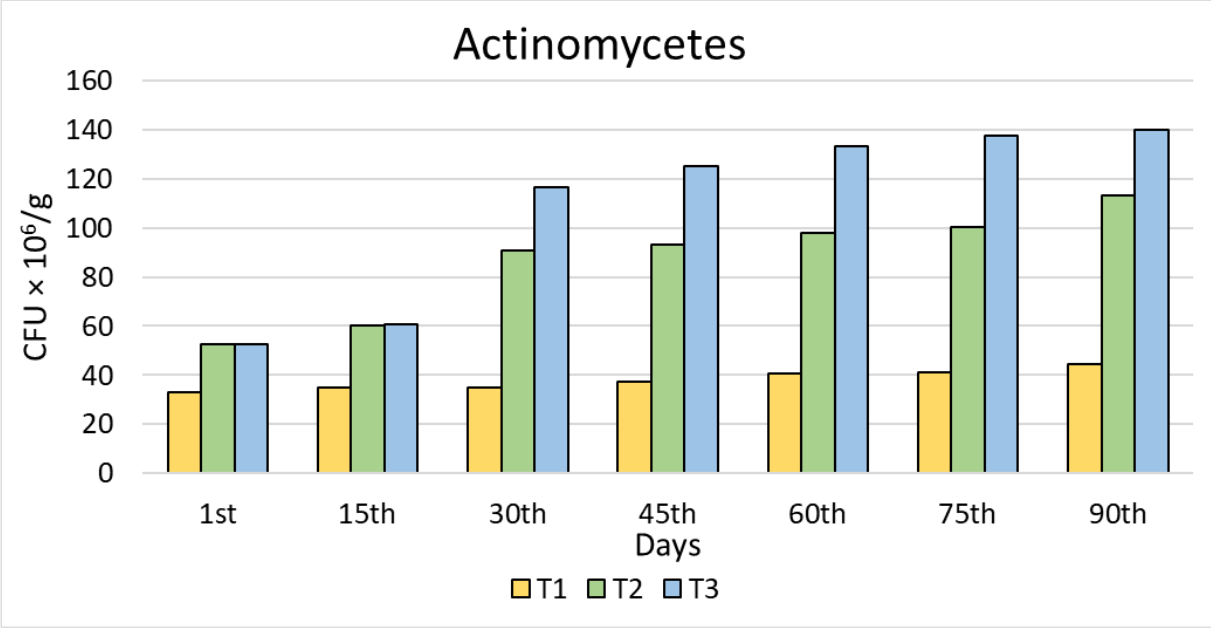

Figure 10. Presence of actinomycetes in the treatments

4.2.5. *Pseudomonas*

Table 8. Presence of *Pseudomonas* in the treatments

Days	T1(CFU×10⁶/g)	T2(CFU×10⁶/g)	T3(CFU×10⁶/g)
1st	43.04	53.05	53.05
15th	44.68	64.82	64.83
30th	47.36	68.43	75.01
45th	49.41	74.21	86.48
60th	50.81	86.08	111.24
75th	52.98	97.98	128.76
90th	54.05	106.31	134.58

The number *of Pseudomonas* found in coffee waste-polluted soil (T1) was 43.04 CFU × 10^6/g. The concentration of *Pseudomonas* did not change significantly when the contaminated soil was left untreated (54.05 CFU × 10^6/g) as shown in Table 8. The concentration of *Pseudomonas* in pre-treated coffee waste rose from 53.05 to 106.31 CFU × 10^6/g after 90 days of composting (T2), and from 53.05 to 134.58 CFU × 10^6/g after 90 days of vermicomposting (T3) as shown in Fig. 11. Composting (T2) and vermicomposting (T3) *Pseudomonas* levels are also quickly increasing.

4.2.6. Nitrogen Fixing Micro-organism

Table 9. Presence of Nitrogen fixing micro-organisms in the treatments

Days	T1(CFU×10⁶/g)	T2(CFU×10⁶/g)	T3(CFU×10⁶/g)
1st	1.57	1.98	1.99
15th	1.82	2.08	2.78
30th	1.94	2.78	3.98
45th	2.02	3.94	5.13
60th	2.54	4.08	5.99
75th	2.86	4.89	6.58
90th	2.98	5.03	7.87

In coffee waste-polluted soil (T1), the quantity of nitrogen-fixing micro-organisms was 1.57 CFU × 10^6/g as shown in table 9. When the polluted soil was left untreated (2.98 CFU × 10^6/g), the nitrogen-fixing micro-organism concentration did not change considerably. After 90 days of composting (T2), the nitrogen-fixing micro-organism content in pre-treated coffee waste increased from 1.98 to 5.03 CFU × 10^6/g, and after 90 days of vermicomposting, it increased from 1.99 to 7.87 CFU × 10^6/g (T3) as shown in fig 12. The levels of composting (T2) and vermicomposting (T3) nitrogen-fixing micro-organisms are also rapidly rising.

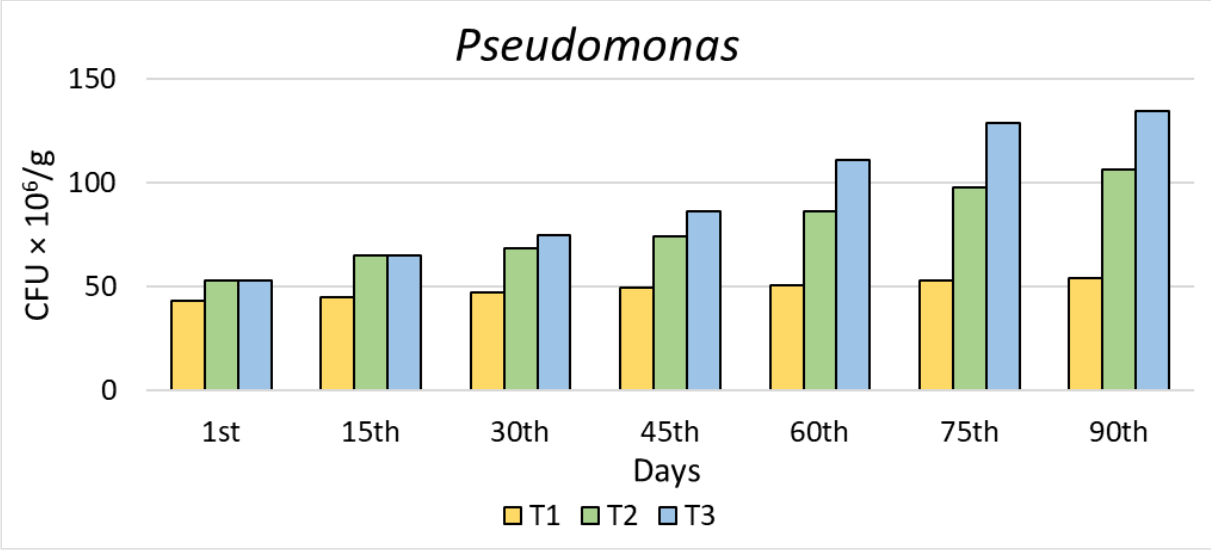

Figure 11. Presence of *Pseudomonas* in the treatments

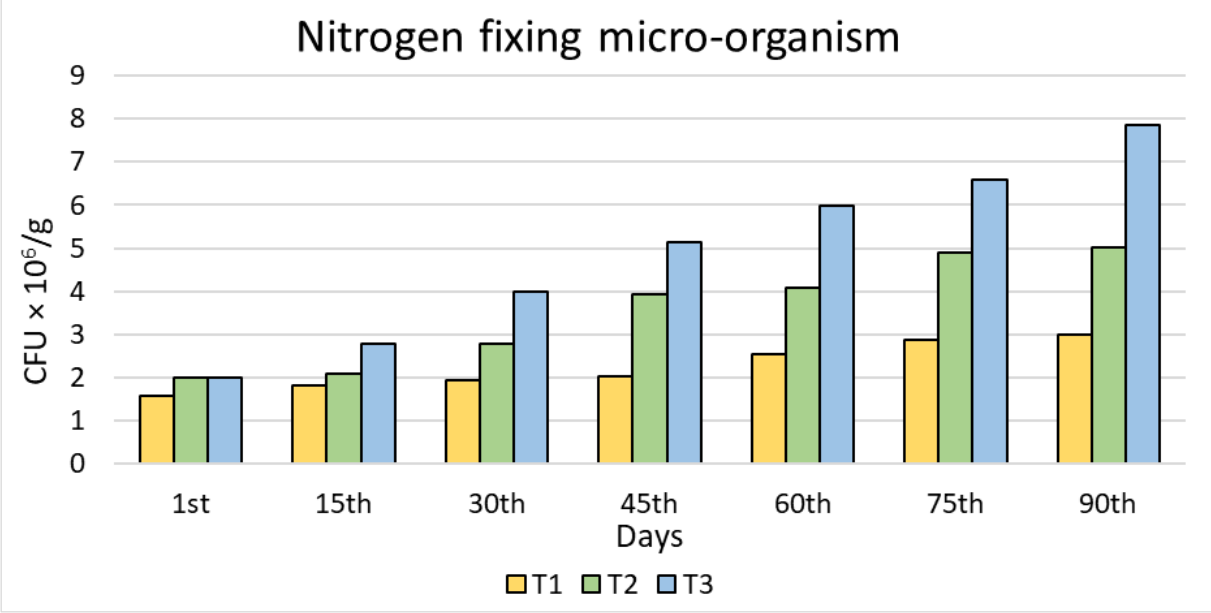

Figure 12. Presence of Nitrogen fixing micro-organisms in the treatments

4.2.7. Phosphate Solubilizers

In coffee waste-polluted soil (T1), there were 25 CFU × 10^6/g of phosphate solubilizers detected. When the polluted soil was left untreated (28.84 CFU × 10^6/g), the phosphate solubilizer concentration did not significantly alter as shown in table 10. After 90 days of composting (T2), the phosphate solubilizers in pre-treated coffee waste increased from 27.44 to 32.86 CFU × 10^6/g, and after 90 days of vermicomposting, the concentration increased from 27.54 to 39.17 CFU × 10^6/g (T3) shown in fig 13. Phosphate solubilizer levels in composting (T2) and vermicomposting (T3) are also on the rise.

4.2.8. Starch Hydrolyzing Micro-organism

In coffee waste-polluted soil (T1), the number of Starch hydrolyzing Micro-organism discovered was 4.86 CFU × 10^6/g as shown in table 11. When the polluted soil was left untreated (7.31 CFU × 10^6/g), the concentration of Starch hydrolyzing Micro-organism did not change considerably. After 90 days of composting (T2), the Starch hydrolyzing Micro-organism content of pre-treated coffee waste increased from 7.26 to 20 CFU × 10^6/g, and after 90 days of vermicomposting, it increased from 7.28 to 25.38 CFU × 10^6/g (T3) shown in fig 14. The levels of Starch hydrolyzing micro-organisms in composting (T2) and

vermicomposting (T3) had also risen rapidly.

Table 10. Presence of phosphate solubilizers in the treatments

Days	T1(CFU×10^6/g)	T2(CFU×10^6/g)	T3(CFU×10^6/g)
1st	25	27.44	27.54
15th	25.9	28.18	30.05
30th	26.34	29.01	32.49
45th	26.99	29.89	35.13
60th	27.34	30.01	36.83
75th	28.01	31.49	38.83
90th	28.84	32.86	39.17

Table 11. Presence of Starch hydrolyzing Microorganism in the treatments

Days	T1(CFU×10^6/g)	T2(CFU×10^6/g)	T3(CFU×10^6/g)
1st	4.86	7.26	7.28
15th	5.08	14	14.01
30th	5.98	17.73	17.83
45th	6.01	18.93	19.04
60th	6.63	19.01	20.96
75th	6.93	19.43	23.89
90th	7.31	20	25.38

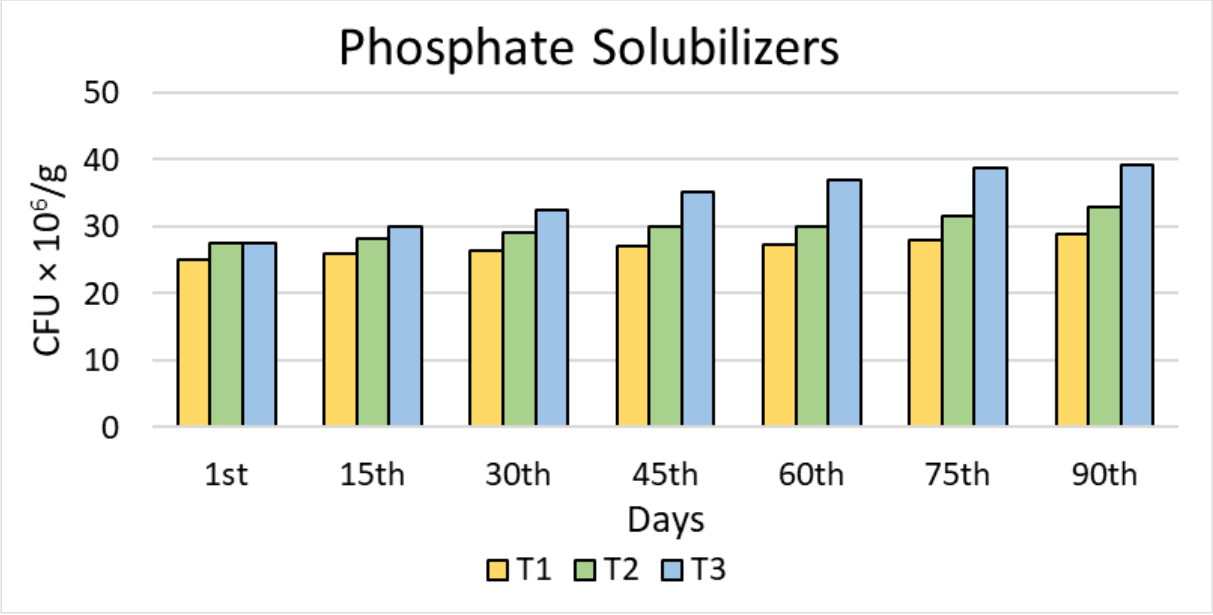

Figure 13. Presence of phosphate solubilizers in the treatments

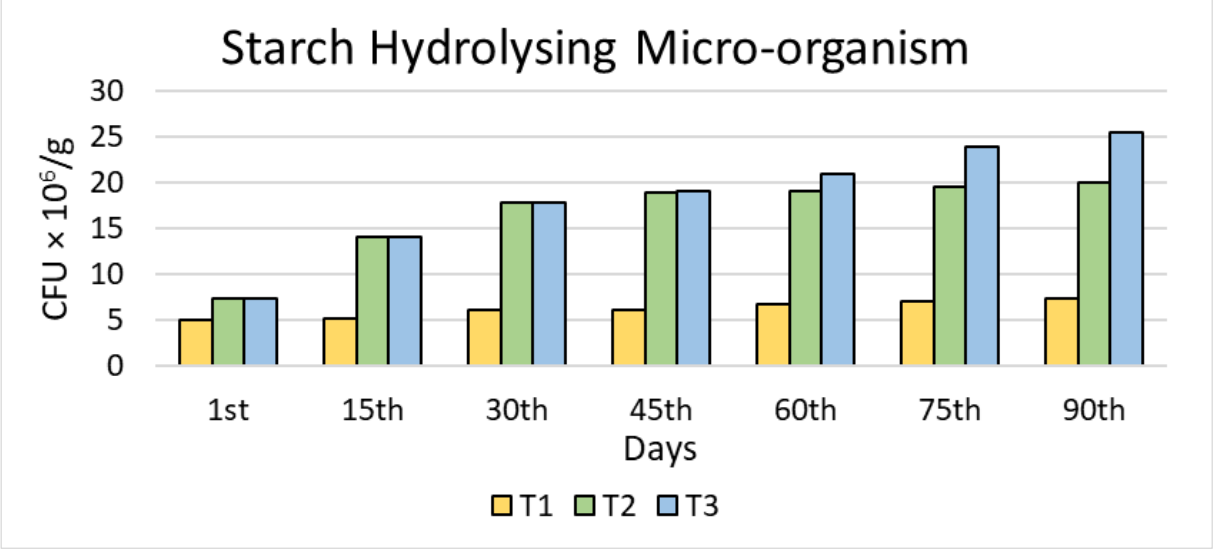

Figure 14. Presence of Starch hydrolyzing Microorganism in the treatments

4.2.9. Azotobacter

The amount of Azotobacter identified in coffee waste-polluted soil (T1) was 25 CFU × 10^6/g as shown in table 12. The concentration of Azotobacter did not change significantly when the contaminated soil was left untreated (32.01 CFU × 10^6/g). The Azotobacter concentration of pre-treated coffee waste rose from 28.45 to 42.61 CFU × 10^6/g after 90 days of composting (T2), and from 28.88 to 50.06 CFU × 10^6/g after 90 days of vermicomposting (T3) shown in fig 15. Azotobacter levels in composting (T2) and vermicomposting (T3) were also quickly increasing.

4.2.10. Pectinolytic Micro-organism

In coffee waste-polluted soil (T1), the quantity of Pectinolytic micro-organisms found was 2.33 CFU × 10^6/g as shown in table 13. When the polluted soil was left untreated (4.01 CFU × 10^6/g), the concentration of pectinolytic micro-organisms did not change appreciably. After 90 days of composting (T2), the pectinolytic micro-organism concentration in pre-treated coffee waste increased from 3.15 to 8.06 CFU × 10^6/g, and after 90 days of vermicomposting, it increased from 3.59 to 11.77 CFU × 10^6/g (T3) shown in fig 16. The levels of pectinolytic micro-organisms in composting (T2) and vermicomposting (T3) were likewise rapidly rising.

Table 12. Presence of Azotobacter in the treatments

Days	T1(CFU×10^6/g)	T2(CFU×10^6/g)	T3(CFU×10^6/g)
1st	25	28.45	28.88
15th	26.08	34.89	34.96
30th	27.43	35.34	37.13
45th	28.76	36.73	40.93
60th	30.89	39.01	43.81
75th	31.43	40.98	45.93
90th	32.01	42.61	50.06

Table 13. Presence of pectinolytic micro-organisms in the treatments

Days	T1(CFU×10^6/g)	T2(CFU×10^6/g)	T3(CFU×10^6/g)
1st	2.33	3.15	3.59
15th	2.89	5.49	5.53
30th	2.99	6.01	7.32
45th	3.08	6.86	8.98
60th	3.53	7.01	9.01
75th	3.89	7.26	10.99
90th	4.01	8.06	11.77

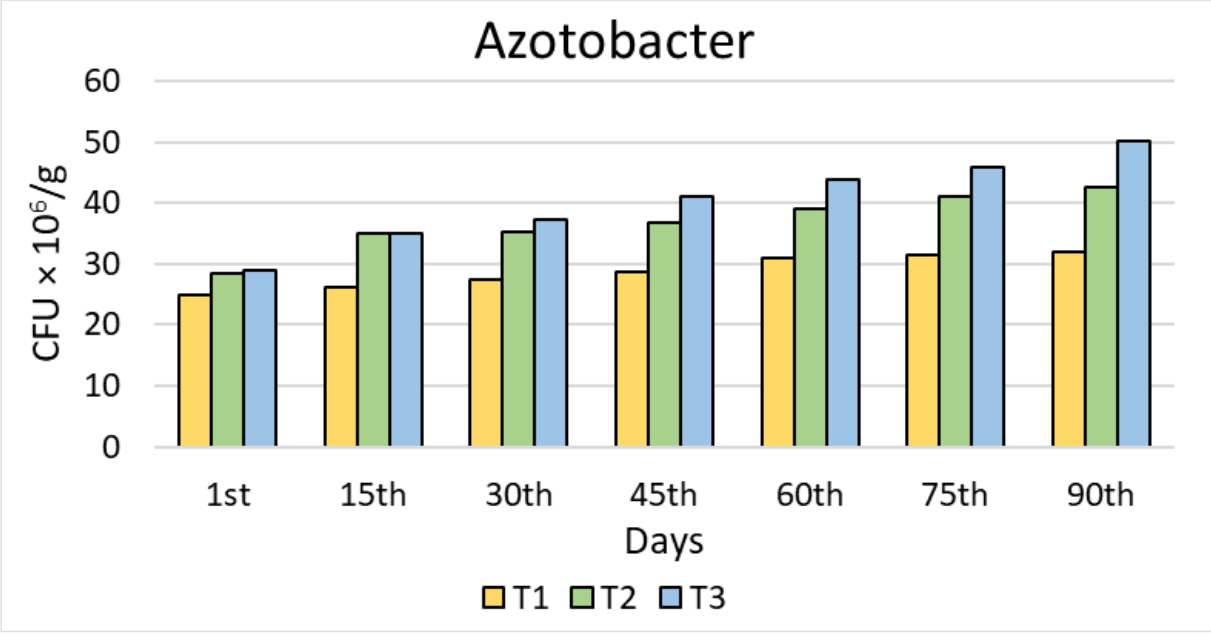

Figure 15. Presence of Azotobacter in the treatments

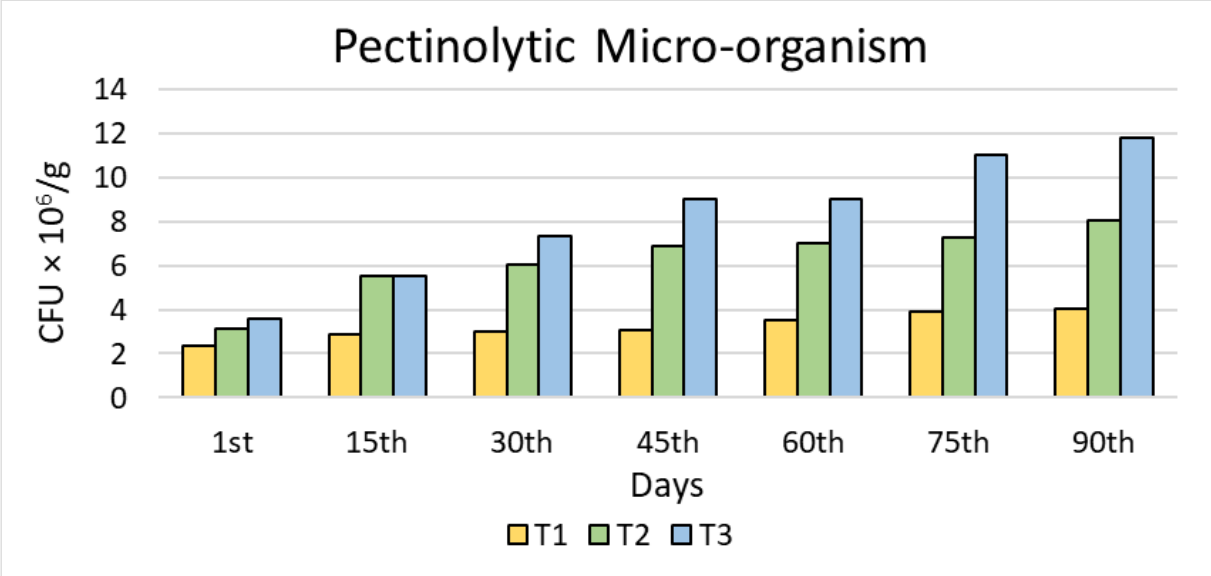

Figure 16. Presence of pectinolytic micro-organisms in the treatments

4.2.11. Chitinolytic Micro-organism

Table 14. Presence of chitinolytic micro-organisms in the treatments

Days	T1(CFU×10^6/g)	T2(CFU×10^6/g)	T3(CFU×10^6/g)
1st	0.66	1.82	1.83
15th	0.85	3.19	3.19
30th	0.96	3.98	4.64
45th	1	4.41	6.78
60th	1.12	5.58	8.35
75th	1.23	5.93	9.09
90th	1.25	6.13	10.03

In coffee waste-polluted soil (T1), there were 0.66 CFU × 10^6/g of chitinolytic micro-organisms as shown in table 14. When the polluted soil was left untreated (1.25 CFU × 10^6/g), the concentration of chitinolytic micro-organisms did not significantly alter. After 90 days of composting (T2), the concentration of chitinolytic micro-organisms in pre-treated coffee waste increased from 1.82 to 6.31 CFU × 10^6/g, and after 90 days of vermicomposting, it increased from 1.83 to 10.03 CFU × 10^6/g (T3) shown in fig 17. The levels of chitinolytic micro-organisms in composting (T2) and vermicomposting (T3) were rapidly rising as well.

The colony- forming units and the microbial load of bacteria, fungus, yeast and actinomycetes were comparatively less in polluted soil whereas the pre-treatment increased the microbial load of all plant growth- promoting microorganisms such as *Pseudomonas sp*., nitrogen-fixing microorganisms, phosphate solubilizers, starch hydrolytic microorganism, Azotobacter, pectinolytic microorganism and chitinolytic microorganism. The vermicomposting significantly increased the microbial load of useful microorganisms (T3).

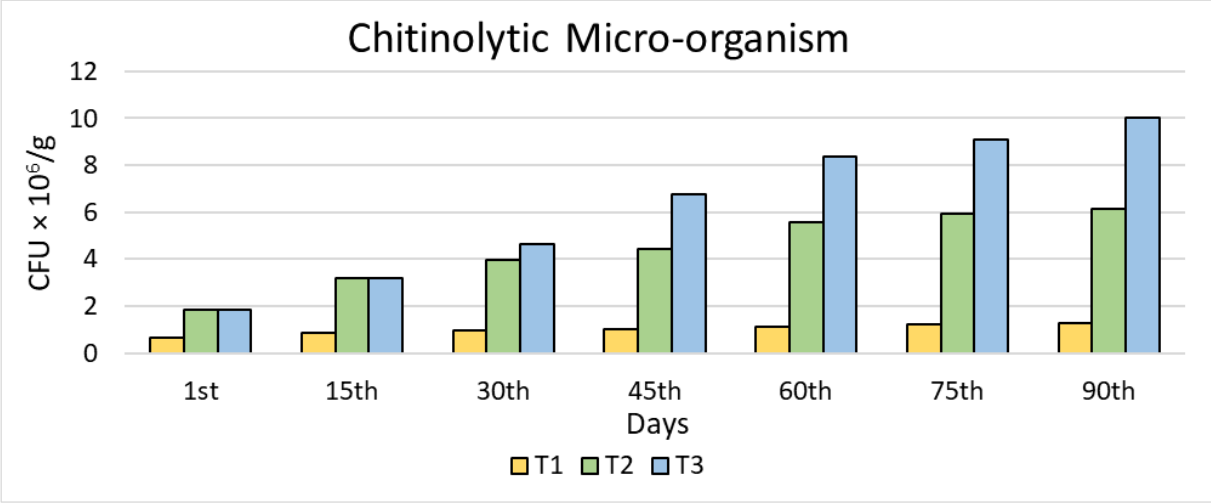

Figure 17. Presence of chitinolytic micro-organisms in the treatments

4.3. Statistical Analysis

Statistical analysis of all the parameters was carried out. Conclusions were made using the arithmetic mean of the triplicate. Standard deviation was also calculated.

4.3.1 Comparative Analysis of Chemical Content

Table 15. Presence of chemical content in treatment after 90 days

Treatment	Caffeine(mg/g)	Chlorogenic acid(mg/g)	Tannins(mg/g)
T1	1.25	0.66	0.35
T2	0.11	0.065	0.03
T3	0.05	0.0523	0.02

The soil polluted with coffee waste was found to be acidic. The acidity of the soil affects the overall physio-chemical and biological properties of the soil. The acidic soil can have poor soil physical properties such as low structural stability and permeability. At low pH the risk of deficiency of base nutrients such as calcium, magnesium and potassium increases, and the solubility of molybdenum and phosphorus compounds decreases as shown in table 15.

The soil polluted with coffee waste was found to have a higher concentration of phytotoxic chemicals such as caffeine, chlorogenic acid and tannins. Caffeine is a xanthine derivative (1,3, 7-trimethyl xanthine). Its presence in higher concentrations in soil has an inhibitory effect on the growth of plants. Arabidopsis seedlings showed growth retardation and early senescence when exposed to caffeine. Chlorogenic acid is a group of polyphenols. Both coffee pulp and coffee husk contain chlorogenic acid and hence the soil polluted with them also contained the chemical. Chlorogenic acid showed an inhibitory effect on the germination of *Artemisia herba* alba seedlings. Tannins are groups of polyphenols. The leachate of tannin occurs in soil polluted with coffee processing waste. Tannins have low biodegradability and will accumulate in the food chain. The pre-treatment of coffee processing and vermicomposting successfully reduced all three reported phytochemicals.

The graph in Fig 18 clearly depicts that treatment three which was treated as vermicompost has the lowest amount of phytotoxic chemical content comparatively. Only 0.05% of chemical content is determined which is approximately 1.2% lower than treatment 1.

4.3.2. Comparative Analysis of Microorganism Content

Soil is a living system acting as a reservoir of water and nutrients. It is a dynamic entity in which the interaction of physical, chemical and biological components takes place continuously. The quality of the soil is essential for sustainable agriculture. Soil physical properties such as texture play an important role in maintaining the quality of the soil since it in turn affects the infiltration rate and cation exchange capacity of the soil. Vermicomposting greatly improves the texture of the soil. The extreme pH decreases microbial activity in the soil which in turn leads to decreased organic carbon decomposition, and nitrification. The pre-treatment of the coffee processing waste with cow dung and the subsequent vermicomposting resulted in the production of vermicompost with a slightly alkaline pH (7.25). The general microbial load was found to get increased during the course of vermicomposting. The plant growth- promoting microbes and their load significantly declined in the soil polluted with coffee processing waste. The pre-treatment of the waste and the subsequent composting changed the scenario, leading to the increased microbial load as shown in table 16.

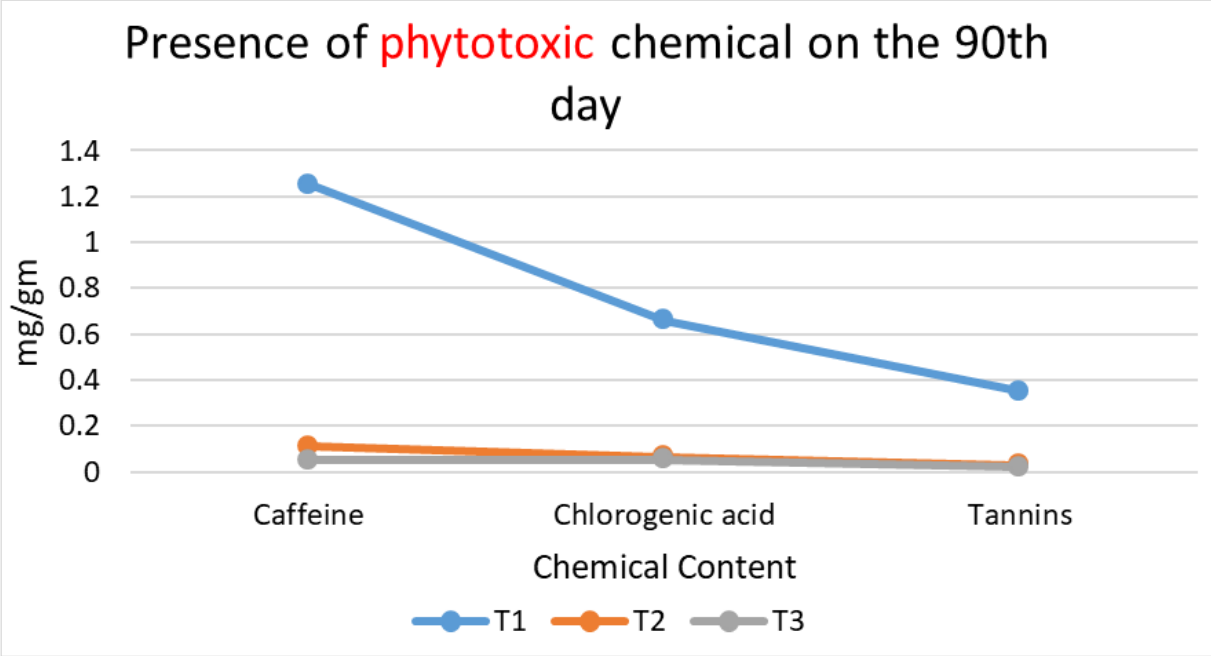

Figure 18. Presence of chemical content in treatment

Table 16. Presence of microorganisms in the treatment (CFU×10^6/g)

	Bacteria	Fungus	Yeast	Actino mycetes	*Pseudomonas*	Nitrogen fixing microorganism	Phosphate solubilizers	Starch hydrolysing microorganism	Azoto bacteria	Pectinolytic microorganism	Chitinolytic microorganism
T1	67.01	24.5	33.31	44.38	54.05	2.98	28.84	7.31	32.01	4.01	1.25
T2	121.42	34.25	110.3	113.49	106.31	5.03	32.86	20	42.61	8.06	6.13
T3	132.64	52.03	131.8	140.19	134.58	7.87	39.17	25.38	50.06	11.77	10.03

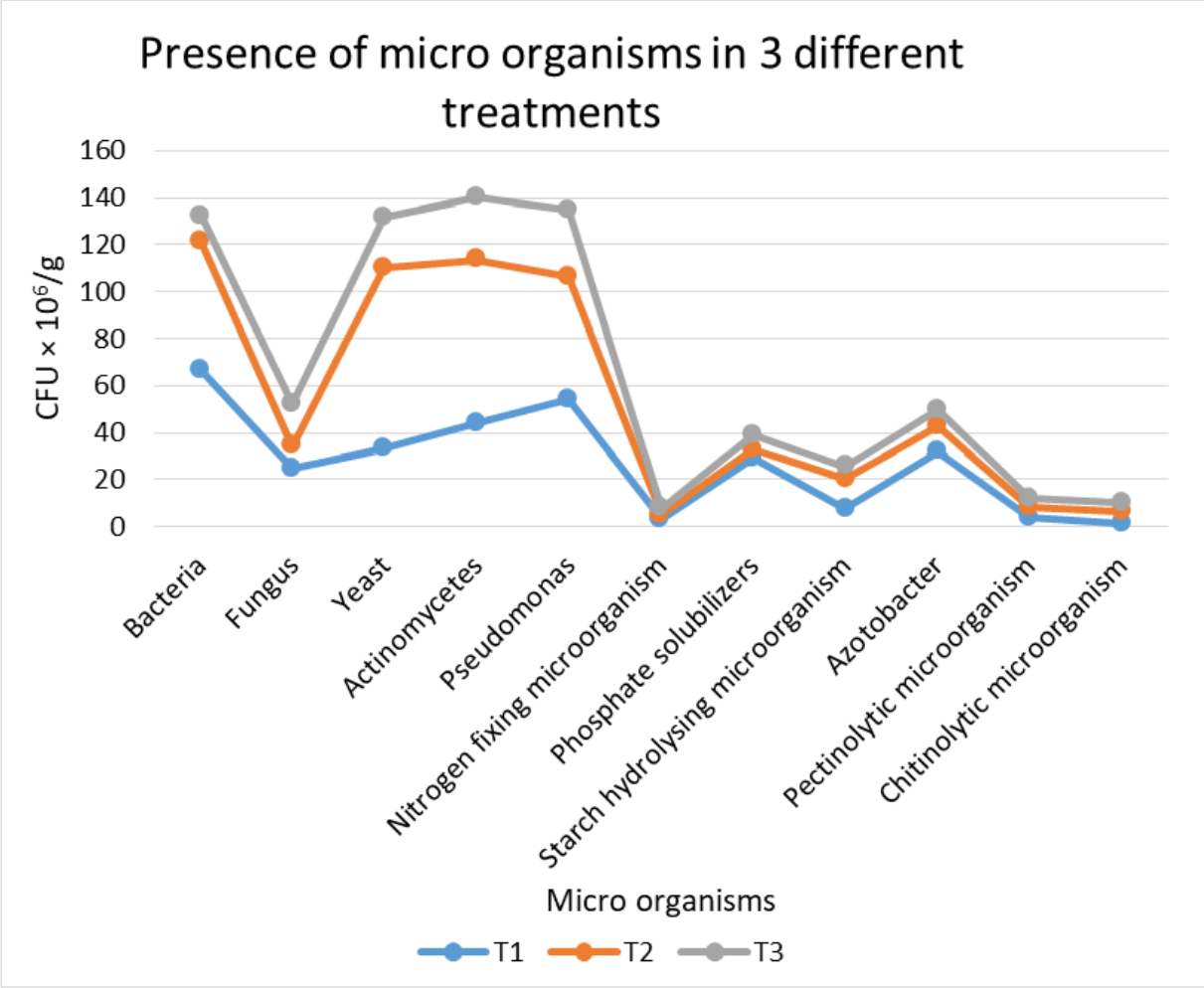

Figure 19. Presence of microorganism 3 different treatments (CFU×10^6/g)

The statistical analysis of the research revealed an increase in the content of micro-organisms in the treated soil as shown in fig 19. The vermicompost promoted the growth of microbial life and thereby making the soil aid for better agricultural manure. On the 90th day the presence of micro-organism content in the third treatment was found to be comparatively higher than the other two. The presence of actinomycetes was found to be highest in the soil at about 140 CFU × 10^6/g and the nitrogen-fixing organism was about 7 CFU × 10^6/g which was found to be the least. However, all the micro-organisms showed a drastic increase in the content in the final analysis.

5. Conclusions

This study revealed that both coffee pulp and coffee husk can be very well used as substrates for vermicomposting using *Eudrilus eugiensis*, an exotic earthworm. The direct dumping of coffee waste in landfill generates severe ecotoxicological problems and destroys the plant growth-promoting microorganisms in the soil. Vermicomposting and composting successfully reduced the concentration of phytotoxic chemicals such as caffeine, chlorogenic acid and tannins which has been proven by this research. The microbial load of the general microorganism and plant growth- promoting microorganisms were significantly increased in the vermicompost as examined in the sample treatment. Thus, it can be concluded that instead of directly dumping the waste into soil it can be converted into nutrient-rich vermicompost and can be recycled in the coffee estate which can contribute highly to recycling the coffee by-products efficiently without polluting the soil.

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