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# Composting of Digestate from Anaerobic Tannery Wastes Biogas for Plant Nutrition

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## Abstract

The disposal and treatment of wastes generated has been the main challenges facing the leather industry. Anaerobic co-digestion of those wastes for production of biogas is one of the promising alternatives if it is accompanied with appropriate disposal of the digestate produced through process. One of the best existing options for digestate removal is recycling it in agriculture after conversion in to stable and nontoxic form. Therefore, this work aimed to determine quality compost from digistate of tannery wastes biogas by co-composting with saw dust and cow dung. Valuable micro and macro nutrients were obtained in both raw digestate and matured compost. High pH, low seed germination index were also analyzed for raw digestate which can may hinder direct use of the digistate in the agricultural field. After composting an improvement in both physiochemical and biological properties of digestate was obtained compared to matured compost standards. Significant improvement in pH, toxicity to seed of seed, water soluble phenol and C:N ratio were found at the end of composting ($p > 0.05$). But no significant difference in soluble nitrate, TOC contents among *Trichoderma viride, Aspergillus niger* and control treatments. More significantly TKN evolution was observed for fungi inoculated treatments ($p < 0.05$). During digestate composting fungi inoculations (*Trichoderma viride* and *Aspergillus niger*) improve some physic chemical and biological parameters to matured compost standards even though statistically still insignificant than control treatments for most parameters

***Keywords:*** *Lignocellulolytic Fungi; Digestate; Co-Composting; Quality Compost*

## Introduction

Tanning is one of the oldest industries in the world and the modern tanning industry in Ethiopia was started in mid of 1920s. More than half the world's tanning activity occurs in low and middle income countries because of the relatively inexpensive cost of labor and raw input materials [1]. Ethiopia is one of the most densely populated countries in the world with more than 100million estimated populations. It is highly gifted with a large number of livestock populations. This high volume and diversified type of cattle population in addition to cheap labor makes the country one of the best places in the world to engage in tanning. Leather has been at the core of Ethiopia's economy since many centuries besides producing a huge quantity of wastes every day. Among all the industrial wastes tannery effluents are ranked as the highest pollutants [2]. Tannery industry spreads chemical, bad smell, liquid and solid waste garbage that pollute air, soil and water [2,3]. In Ethiopia all pre-tanning leather alone annually generate 70,104 tonnes of solid waste and 3,393,600$m^3$ wastewater and dispose into the surrounding environment without proper treatment [3].

Anaerobic digestion is one of the promising technologies to reduce environmental burden of tannery wastes with additional production of useful products such as biogas (methane) which is used as good source of energy. Ethiopia is practicing anaerobic digestion to manage wastes generated by tannery industry and to produce value added biogas.

Anaerobic digestion is also an important method to decrease the quantity of organic wastes and COD to be discharged to environment. In Ethiopia [3] were obtained 75% COD removal from bench scale study, through two stage co-digestion of tannery liquid waste with solid waste. Anaerobic digestion technology in addition to COD removal also destroy pathogenic bacteria such as fecal coliforms and enterococcus [4]. On top of this anaerobic technology for biogas production still remains with byproducts to be managed and further treated properly [5] were recommended biogas plant should take inconsideration of its flue gas, odour emissions and the digestate management. Digestate is the byproduct of biogas plant coming from organic wastes wet digestion processes contains un degraded organic waste, microorganism cells and structures formed during digestion as well as some inorganic matter. This is potentially an alternative source of humic material, nutrients and minerals for the agricultural soil [6]. But sometimes direct application of digestate into agricultural soils presents several problems like low concentration of nutrients, high salinity, economic cost of transport and handling and environmental issues such gaseous emissions, nutrient leaching and pathogen spread [7,8]. In spite of this the digestate is rich in macro and micro nutrients which make it suitable for co-composting with other wastes for more stability of the product. Compost produced is good for agricultural fertilizers input and also a good means of digestate removal as well. Digestate co-composting with other wastes has additional beneficial effects such as stabilization of organic matter, elimination of unpleasant odours and reduction of pathogenic microorganisms below threshold limit level [7]. But during digestate composting deficiencies in the indigenous microbial community can leads to a low composting efficiency and consequently affect the matured compost quality [9]. Therefore, microbial inoculums use accelerates the overall composting process even though the benefits of direct microbial inoculation into composting substrates still need further study [10]. Yet, few studies have investigated the composting of digestate with addition of lignocellulolytic fungi inoculums. Therefore, study was conducted to determine compost quality from anaerobic digestate derived from tannery wastes biogas by co-composting with cow manure, saw dust and further using lignocellulolytic fungi inoculums.

## Materials and Methods

### Sources of fungi inoculums and digestate

*Trichoderma viride and Aspergillus niger* isolates were obtained from department of Microbial, Cellular and Molecular Biology, College of Natural Sciences, Addis Ababa University. A solid digestate obtained after biogas production from anaerobic co- digested liquid tannery waste with solid tannery waste. For composting experiment only solid state digestate was used. Digestate from biogas was a much liquid type. To make this more thickened simple decantation was used to remove excess water.

### Experimental design

After determining recommended compost initial raw materials carbon to nitrogen ratio digestate was mixed with saw dust and cow dung in ratio of 1:1:1 on dry weight basis. Treatments were performed in triplicate (Figure 1).

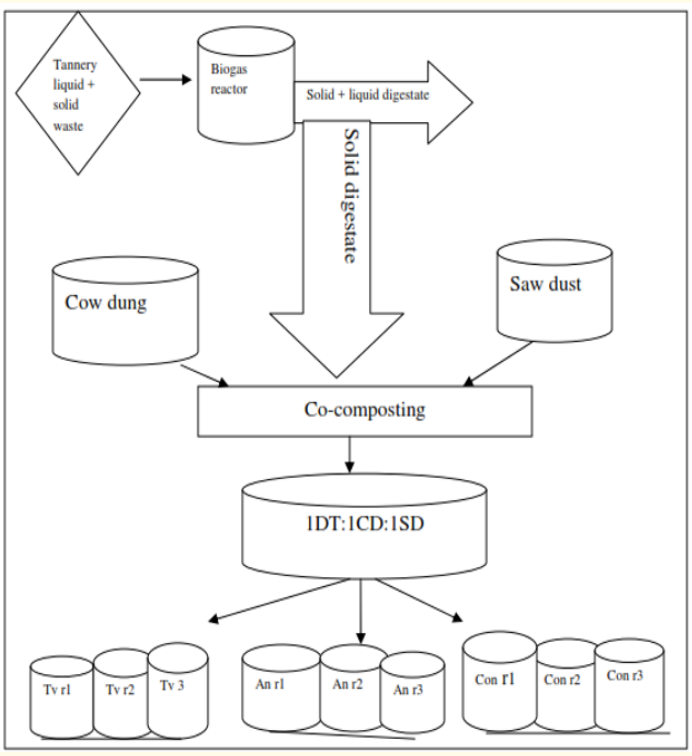


***Figure 1:*** *Experimental Design.*
*Tv: Trichoderma viride treated compost; An: Aspergillus niger treated compost; Con: Control treatments and 1, 2.3 (replication numbers), DT: Digestate; CD: Cow dung; DS: Saw dust and 1:1:1 (mixing ratio).*

The compost input materials were thoroughly mixed and co-composted for 65 days in small bin system in triplicate. 2% in weight of inoculums with respect to the total compost mass was used. Tap water was added to adjust the moisture content to 50%. The compost turning was took place manually about three times a week to ensure the supply of oxygen.

**Physicochemical analysis**

Both the feed stock materials and compost were analyzed for physicochemical parameters using composite samples. Temperature was daily measured using digital thermometer (HANNA instrument, Hi 9055, Portugal). pH and electrical conductivity (EC) were determined using compost to distilled water in a ratio of 1:5 w/v on filtered extract [11]. Total Organic Carbon (TOC) was determined titrimetrically by modified Walkley-Black titration [12]. Total nitrogen determined by Kjeldahl digestion method [13] using Kjeldahl apparatus CABCONCO 141102492, SIEMENS Germany. Total Phosphorus after wet digestion was determined by Olsen method [13]. Water soluble phosphorous and water soluble $NO_3$-N was measured after extraction of 1:5 w/v soil water extraction).Total metal analysis for Zn, Cu, Fe, Mn, Ca, Mg, Na K. Cr and Pb were done after extracting through dry ashing [14] followed by reading on an atomic absorption spectrophotometer AAS, ell 400. Compost germination bioassay was conducted according to method of [15,16] using seed of Soybean (*Glycine max*). For Cellulase activity Assay Carboxymethyl cellulose was used. In detail, one ml of 1% carboxymethylcellulose, one ml of 0.05M of sodium acetate buffer pH 5.5 and 0.2 ml of the crude enzyme were mixed and incubate d at 40°C for sixty minutes. After incubation one milliliter of DNS (dinitrosalicylic acid) reagent was added and well vortexed. The reaction mixture heated for ten minutes in boiling water bath. After cooling absorbance were measured at 560 nm using JENWAY 6705 UV/V, is Spectrophotometer [17].

## Results and Discussions

**Temperature**

Compost temperature profiles are presented in figure 2. Similar temperature profiles trend were observed for all treatments during composting period.

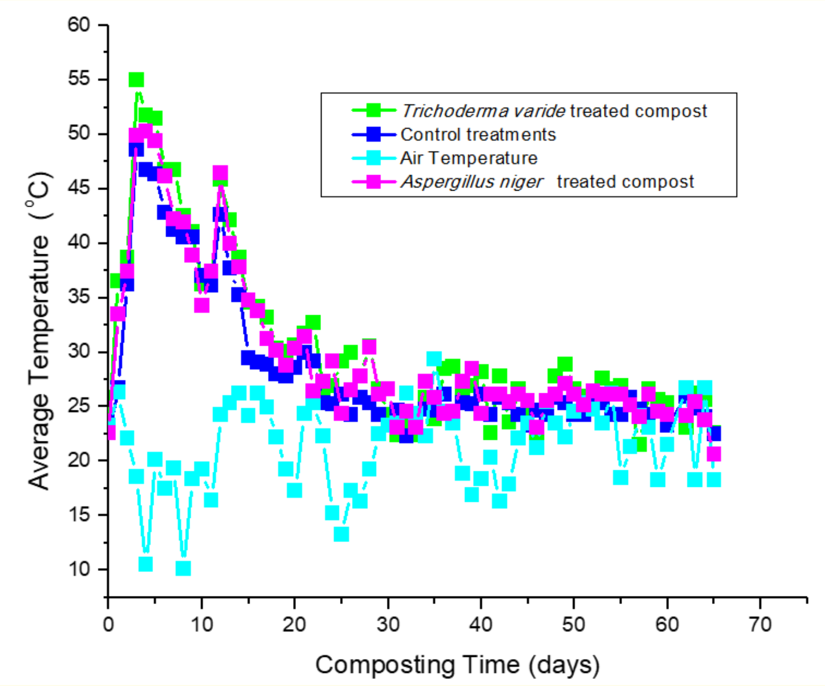


***Figure 2:*** *Compost temperature dynamics.*

No significance difference in temperatures was observed in treatments ($p > 0.05$). Highest temperature was recorded by *Trichoderma viride* (51.4 ± 2.6°C) and lowest temperature (49.38 ± 2.79°C) by control treatments. In all treatments a continuous rapid increment in temperature was observed until maximum temperature above 40°C was achieved at $5^{th}$ day. An increment temperature can be a result of organic matter degradation by microorganisms [18]. As composting proceeded heat generation declined until it will be balanced with the surrounding temperature. At this stage there is reduction of microbial metabolism due to less availability of easily degradable organic matter [8]. The result of present temperature profile was similar with that of temperature profile reported by [8] for digestate composting.

**pH and electrical conductivity**

The pH for saw dust was (5.0 ± 0.05), for raw digestate was (9.234 ± 0.65), for cow dung was (6.67 ± 0.64) and for mixed raw compost input material was (9.12 ± 0.01). pH of the matured compost was 8.5 ± 0.1, 8.3 ± 0.10 and 8.5 ± 0.10 for control, *Trichoderma viride* and *Aspergillus niger* treatment respectively (Figure 3). Decrease in pH was observed until 30$^{th}$ day of composting while small increment was observed on 45$^{th}$ day of composting. This could be related to organic matter potentially available exposed to microbial degradation during turning of the piles. Some authors also found an increase in pH during thermophilic phase of composting and decrease to near neutral pH at maturation phase of composting. This due to formation of humic substances which act as buffers [19]. At the end of composting day we obtained significant decrease in pH in all treatments ($p < 0.05$). This result is similar with pH reported by [7] for co-composting of the solid fraction of anaerobic digestate. The pH values of matured composts were suitable for cultivation of moderately alkaline soil plants.

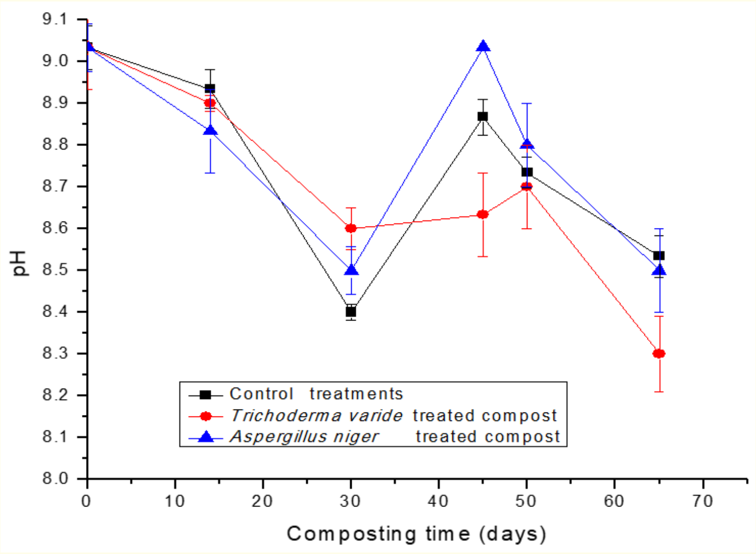


***Figure 3:*** *Compost pH value.*

Electrical Conductivity (EC) was 234.4 ± 0.06 µS cm$^{-1}$, 2.56 ± 0.63 mS cm$^{-1}$, 9.36 ± 0.02 mS cm$^{-1}$ and 3.51 ± 0.70 ms cm$^{-1}$ for saw dust, digestate, cow dung and for mixed waste respectively. High EC value in digestate is a result of high NaCl use during tannery beam house operation. Significant increment in EC was observed for *Trichoderma viride* treatment ($p < 0.05$). Electrical conductivity increased from 253.43 ± 56.62 µS cm$^{-1}$ to 469.9 ± 103.79 µS cm$^{-1}$ 229.77 ± 49.88 µS cm$^{-1}$ to 638.1 ± 256.52 µS cm$^{-1}$ and 547.27 ± 70.16 µS cm$^{-1}$ from initial to final stage of composting for control treatments, *Trichoderma viride* and *Aspergillus niger* respectively (Figure 4). This is due to release of mineral salts and ammonium ions through decomposition of organic matter [20]. Evaporation and condensation as total compost mass decreases leads to EC increment reported by Torres-Climent A., *et al* [8]. In matured compost EC values observed to be higher than most countries recommended guidelines of < 2.00 mScm$^{-1}$ [21,22].

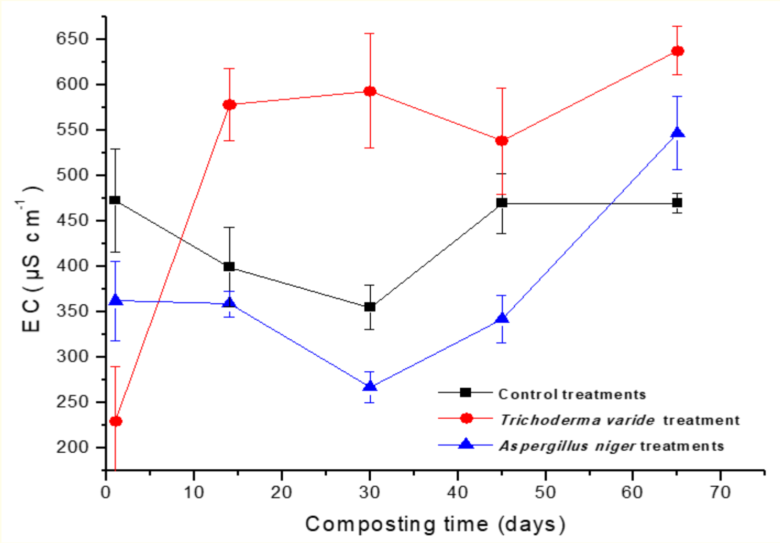


***Figure 4:*** *Change in electrical conductivity during composting period.*

### Water soluble nitrate, water soluble phosphorous and total phosphorous

Significant increment in water soluble nitrate was observed for all treatment during composting days ($p < 0.05$). Water soluble nitrate increased from 222.05 µg $g^{-1}$ to 318.58 µg $g^{-1}$, 282.00 to 364.08 µg $g^{-1}$ and 285.21 to 426.62 µg $g^{-1}$ for control treatments, *Trichoderma viride* and *Aspergillus niger* respectively in the first two weeks (Figure 5). After 65 days of composting water soluble nitrate was further increased to above 630 µg $g^{-1}$. Oxidation of ammonium ($NH_4^+$) or ammonia ($NH_3$) to nitrate ($NO_3$-N) by autotrophic and heterotrophic nitrifying microorganisms in compost [5] and less volatilization due to anoxia [23] can increase water soluble nitrate. Significant loss of $NH_3$ and $N_2O$ during thermophilic stage of composting was reported by [24] which was favored by both high temperature and pH. Further due to frequent turning there was compost fresh materials exposure to microbial oxidation and leads to the release of nitrate [25]. No significant effect was observed on water soluble nitrate dynamics due to fungi inoculums addition ($p > 0.05$) and there was strong cross correlation among treatment in soluble nitrate dynamics ($r > 0.97$).

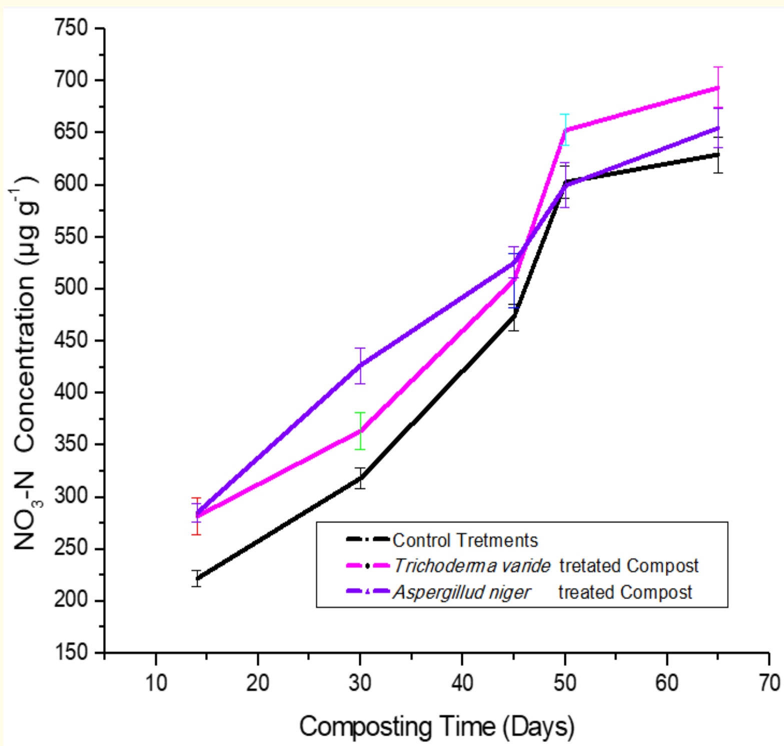


***Figure 5:*** *Change in water soluble nitrate over composting period.*

Total phosphorous and water soluble phosphorous were increased through composting process figure 6 and 7. Two weeks later compost total phosphorus was 6677.42 mg $kg^{-1}$, 6881.72 mg $kg^{-1}$, 6658.77 mg $kg^{-1}$ for the control, *Trichoderma viride* and *Aspergillus niger* treatments. At the end of composting day, total phosphorous significantly increased to 8378.81 mg $kg^{-1}$, 8591.23 mg $kg^{-1}$, 8427.36 mg $kg^{-1}$ for control, *Trichoderma viride* and *Aspergillus niger* treatments ($p < 0.05$). Similarly, two weeks later of composting water soluble phosphorous was 2370 mg $kg^{-1}$, 2710 mg $kg^{-1}$, 2500 mg $kg^{-1}$ for control, *Trichoderma viride* and *Aspergillus niger* treatments respectively. At final day of composting it was further increased to 3738.50 mg $kg^{-1}$, 3615.90 mg $kg^{-1}$, 3726.88 mg $kg^{-1}$ for control, *Trichoderma viride* and *Aspergillus niger* treatments. An in increase in water soluble phosphorous during composting process was attributed to the solubilization of inorganic phosphate by different species of microorganism present in compost raw materials [26].

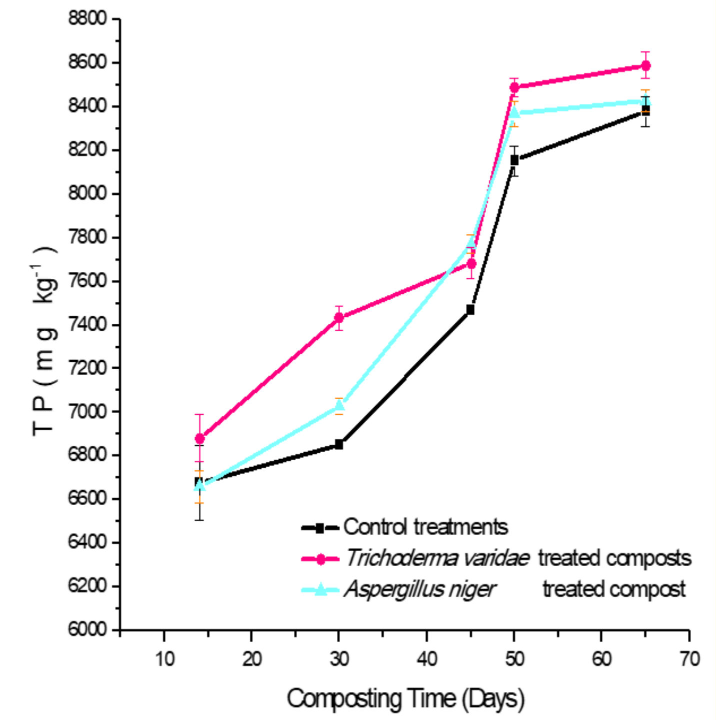


***Figure 6:*** *Change in compost total phosphorous.*

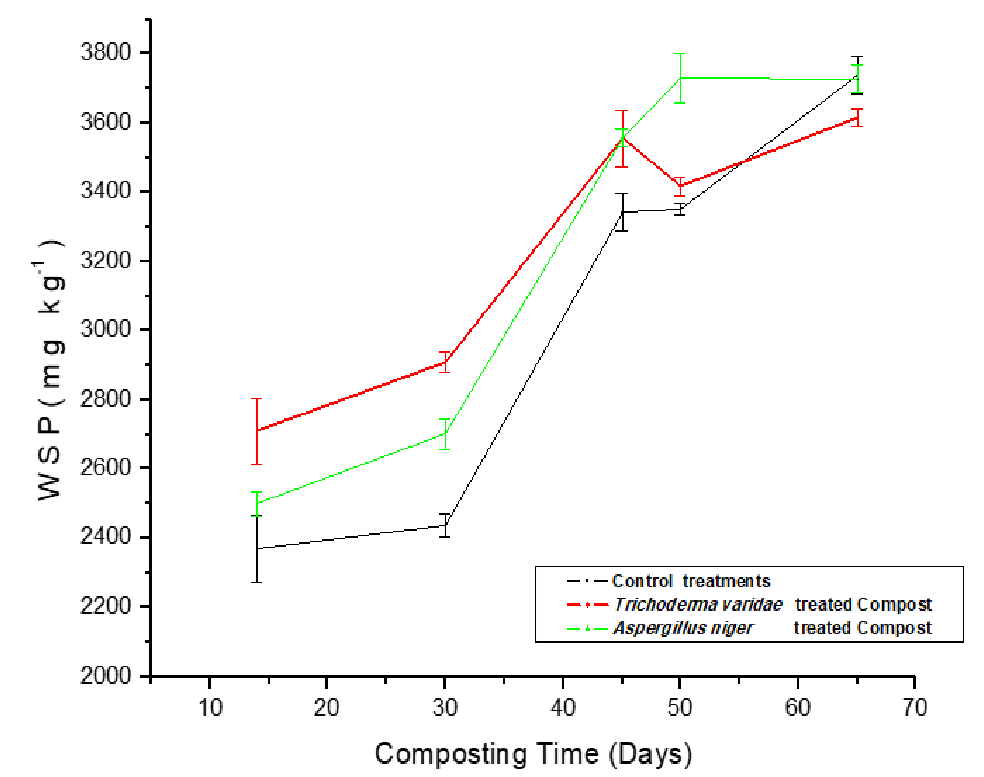


***Figure 7:*** *Change in water soluble phosphorous during composting days.*

**Total organic carbon (TOC), total nitrogen (TK N) and C:N ratio**

High TOC degradation percentages were 29.64 ± 0.50% for *Aspergillus niger*, 27.55 ± 0.60% for *Trichoderma viride* and 24.18.64 ± 0.59% for control treatments (Figure 8). Mineralization of organic matter to $CO_2$ during composting process conveys to decrease in TOC [7,27]. Faster decrease in TOC at initial stage of composting is because of the abundant availability of biodegradable organic matter fractions. Inoculums addition no significantly improves TOC degradation ($p > 0.05$). However, better TOC degradation was recorded for microbial inoculated treatments. Similarly situation was reported by [28] where better TOC degradation was recorded in the microbial inoculated composting compared to the control.

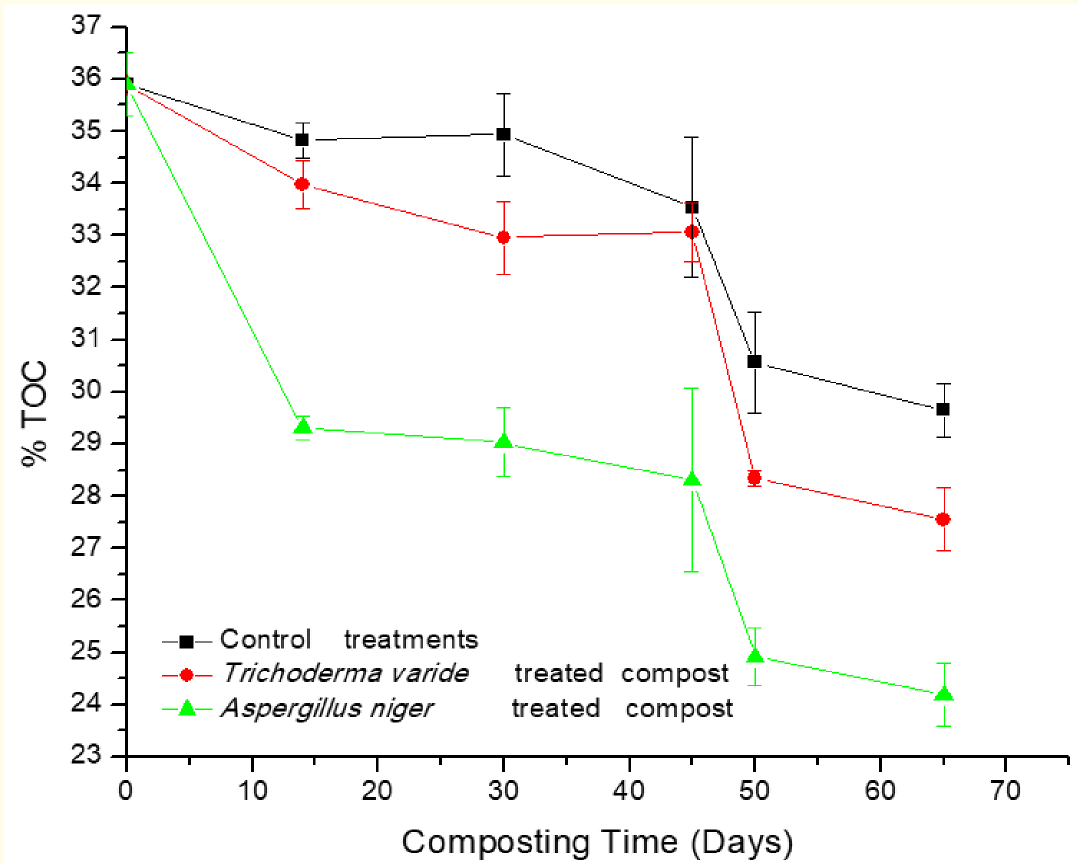


***Figure 8:*** *Dynamics in compost TOC during composting days.*

TKN for Saw dust was (0.13%), for raw digestate was (2.30%), cow dung was (2.12%) and for mixed raw compost input was (2.21%). compost TKN dynamics was presented in figure 9. For matured compost TKN of (2.51%), (2.62%) and (2.75%) were recorded for control, *Aspergillus niger* and *Trichoderma viride* treatment respectively. Compost microbial inoculums addition significant improves evolution of TKN than control treatments ($p < 0.05$). Better TKN evolution was obtained for Increments in total nitrogen from feedstock to matured compost are related to enhancement of nutrient [10] and also due to reduction in weight because of decomposition.

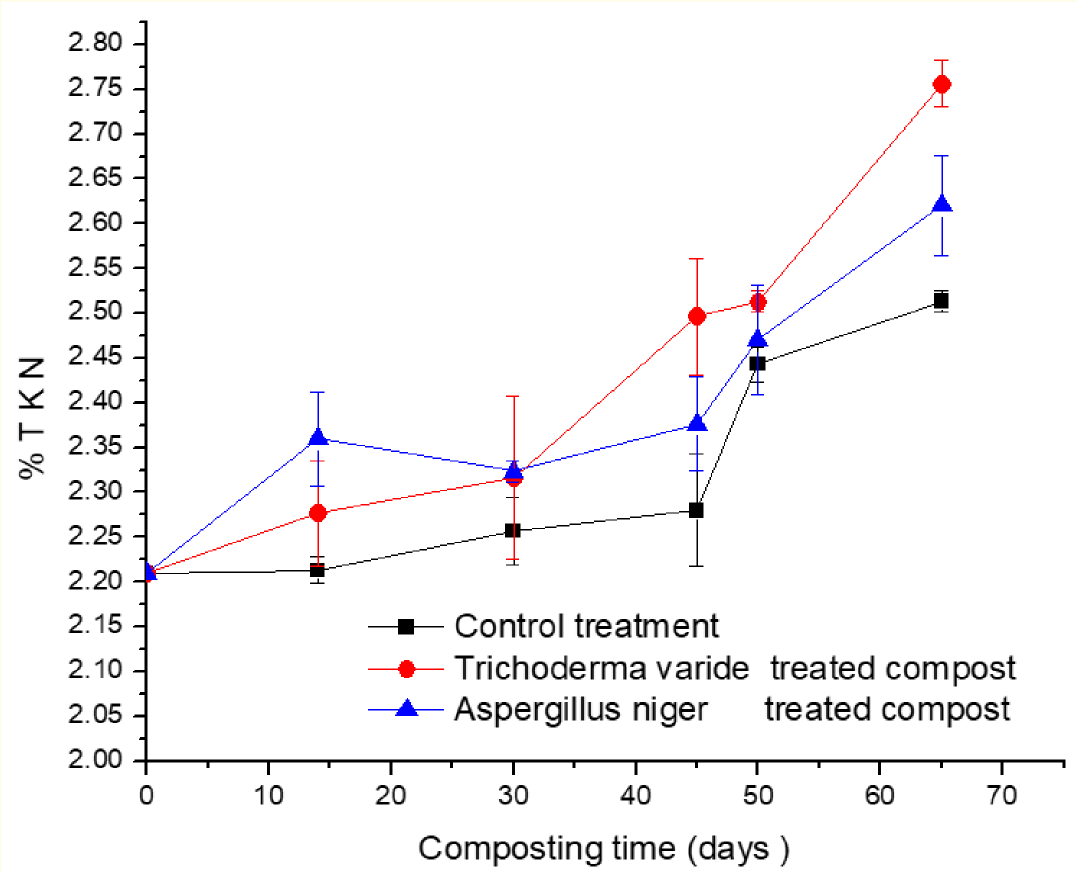


***Figure 9:*** *Change in compost TKN.*

C:N ratio for matured compost significantly lower than feedstocks of compost (P < 0.05). At the end of composting day C:N ratio values of 9.99, 10.75 and 11.79 were recorded for *Trichoderma viride*, *Aspergillus niger* and control treatments (Figure 10). At final stage of compost reduction in C:N ratio was greater for *Trichoderma viride* (38.48%), followed by *Aspergillus niger* (33.80%) and lastly by control treatments (27.34%). After co-composting of the digestate we found matured compost with recommended range of C:N for agricultural field application. Compost is best mature if C:N is < 20, preferable < 10 according to [29] and C:N standard limit for mature composts should be less than 12 was suggested by Bernai M., *et al* [30].

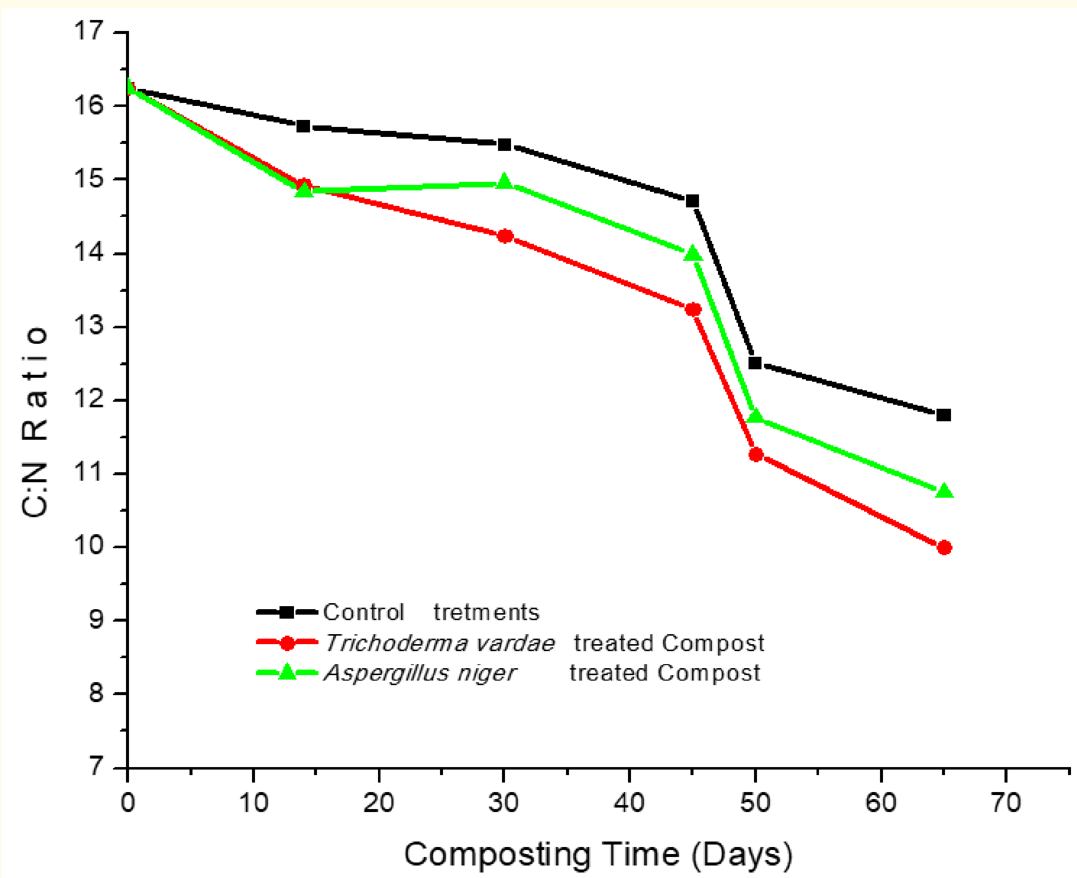


***Figure 10:*** *Compost C:N ratio.*

### Water soluble phenols (WSPH)

Water soluble phenols at the end of composting day were found to be 73.94%, 70.50% and 52.11% for control, *Aspergillus niger* and *Trichoderma viride* in corresponding order. High reduction in WSPH in control treatment could be explained by low availability water soluble phenol precursor due to resistance of organic matter degradation. WSPH content decreased in all treatments over composting period (Figure 11).

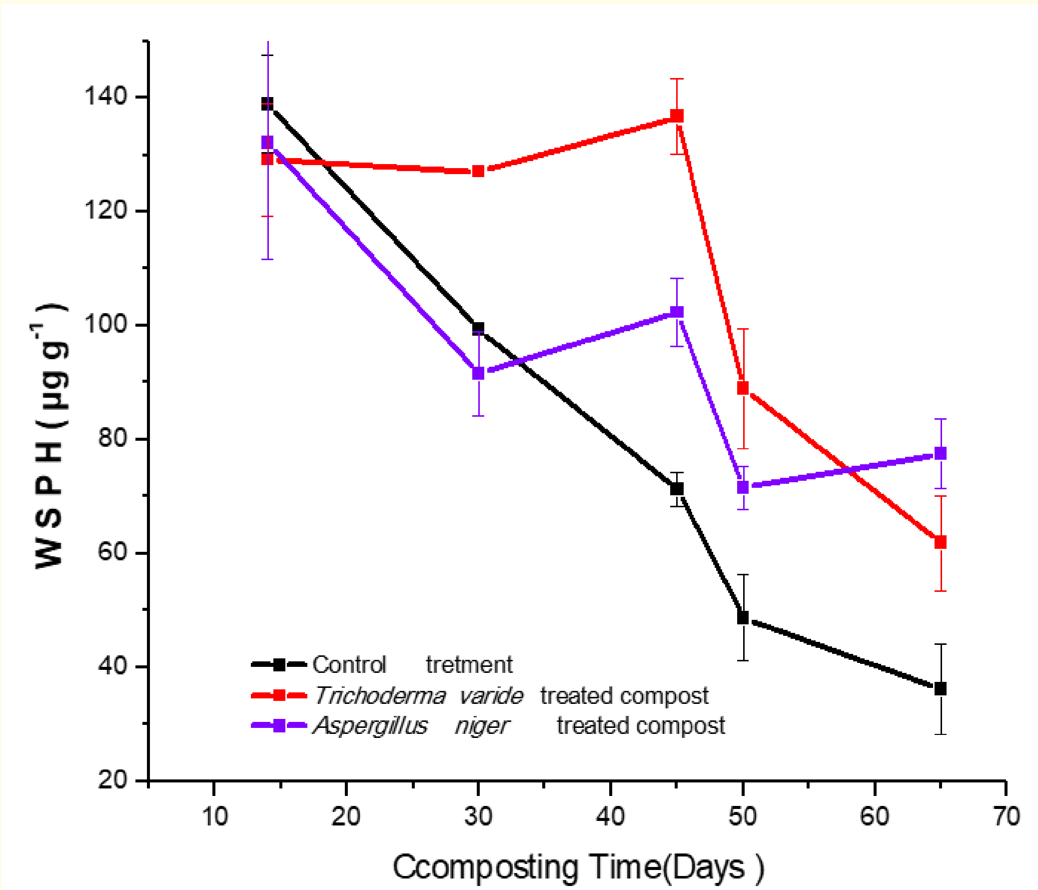


***Figure 11:*** *WSPH content of compost during composting period.*

Phenolic compounds converted to humic substance as composting process going on contributing to reduction in phenol contents at final stage of composting [31]. 93% reduction in WSPH was reported for Olive mill waste composting by Baddi GA., *et al* [32]. According to Spanish legislation compost to be best used as fertilizers if total polyphenols is less than 0.8% was reported by Tortosa G., *et al* [33].

### Total reducing sugar (TRS) and total water soluble carbohydrate (TSS)

Compost Total Reducing Sugar (TRS) and Total water soluble carbohydrate (TSS) dynamics presented in (Figure 12 and 13) respectively. Total reducing sugar after two weeks of composting was 47.99 ± 5.0 µg $g^{-1}$, 137.35 ± 18.23 µg $g^{-1}$ and 117.19 ± 10.43 µg $g^{-1}$. Then on 50$^{th}$ day of composting increased to 138.95 ± 6.30 µg $g^{-1}$, 165.2733 ± 15.54 µg $g^{-1}$ and 133.09 ± 14.38 µg $g^{-1}$ for control treatment, *Trichoderma* *viride* and *Aspergillus niger*. An increment in total water soluble reducing sugar (TRS) at near final stage of composting can be the result of further exposure of organic matter to microbial oxidation. At final stage of composting TRS decreased to 78.30 ± 3.2 μg $g^{-1}$, 52.25 ± 2.8 μg $g^{-1}$, 72.54 ± 5.5 μg $g^{-1}$ for control treatment, *Trichoderma viride* and *Aspergillus niger* respectively. Total water soluble reducing sugar after two weeks of compost was 98.79 ± 5.0 μg $g^{-1}$, 141.69 ± 48.23 μg $g^{-1}$ and 116.45 ± 10.43 μg $g^{-1}$. Then on 30th day of composting increased to 170.12 ± 78.17 μg $g^{-1}$, 196.33 ± 55.10 μg $g^{-1}$ and 176.10 ± 45.85 μg $g^{-1}$ for the control treatment, *Trichoderma viride* and *Aspergillus niger* respectively. Finally at the end of composting day TSR decreased to 123.34 ± 11.97 μg $g^{-1}$, 130.33 ± 7.13 μg $g^{-1}$ and 115.43 ± 10.18 μg $g^{-1}$ for control treatment, *Trichoderma viride* and *Aspergillus niger* respectively. Water soluble sugar reduction at final stage of composting was explained as sugar may fueled to fulfill energy demand of microorganism as described by Lee IB., *et al* [34].

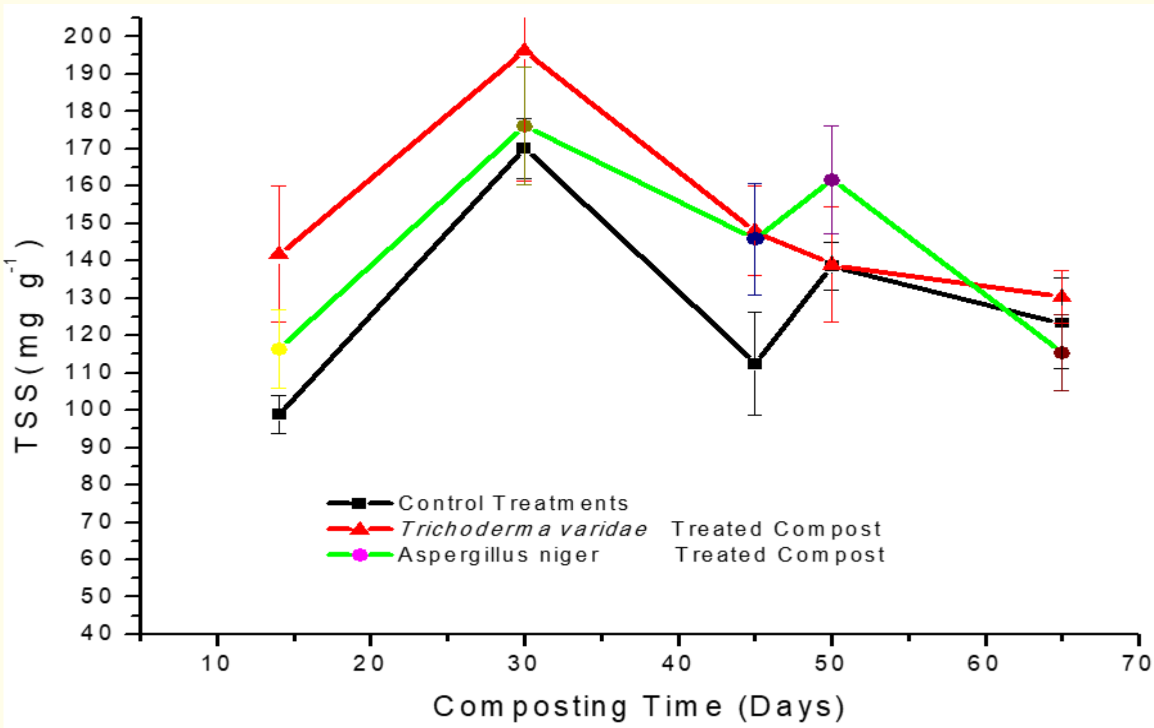


***Figure 12:*** *Compost total soluble sugar.*

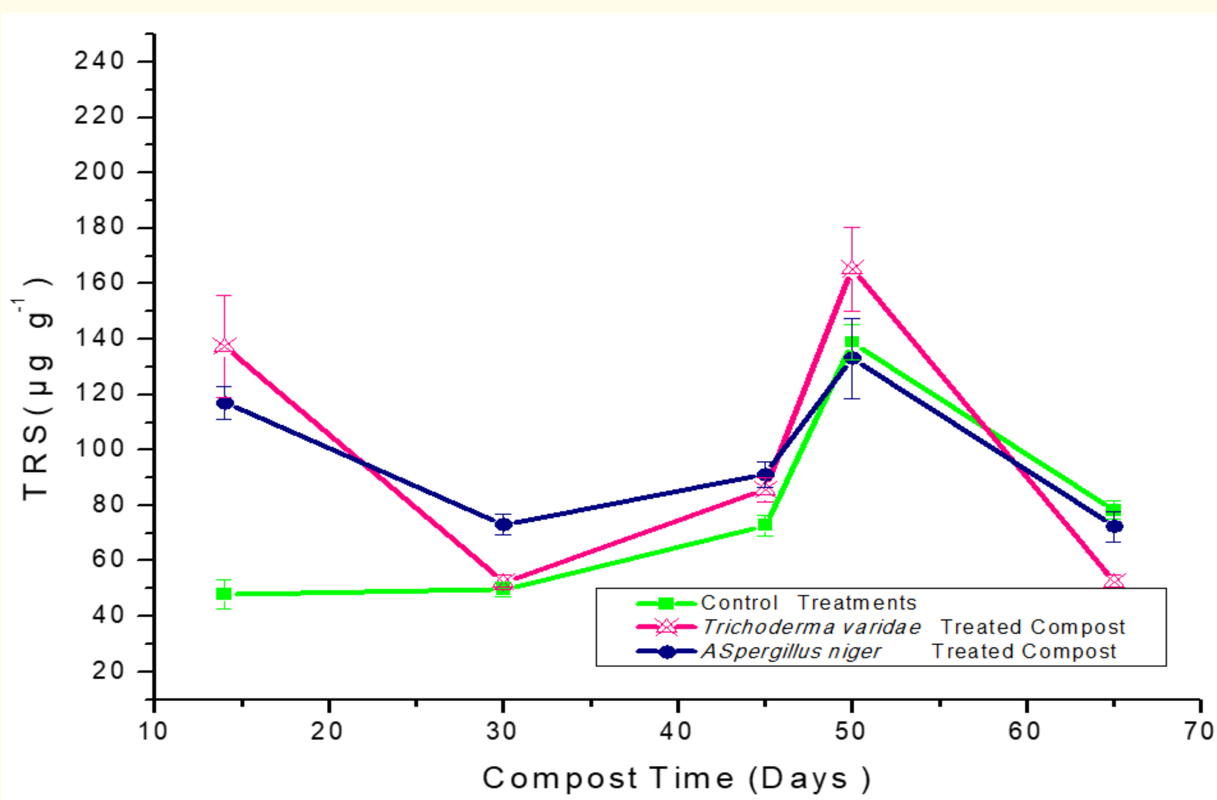


***Figure 13:*** *Compost total reducing sugar dynamics.*

### Cellulase activity assay

Cellulose activity was increased in all treatments for the first two weeks then after continuously decreasing (Figure 14). Similarly [35] was reported decrease in cellulase activity with the advancement of compost product maturity. *Trichoderma viride* has showed the highest cellulose activity through ought the whole composting periods. Un similarly highest cellulose activity was reported for *Aspergillus niger* than *Trichoderma viride* by [36] for composting of empty fruit bunches with Palm oil mill effluent.

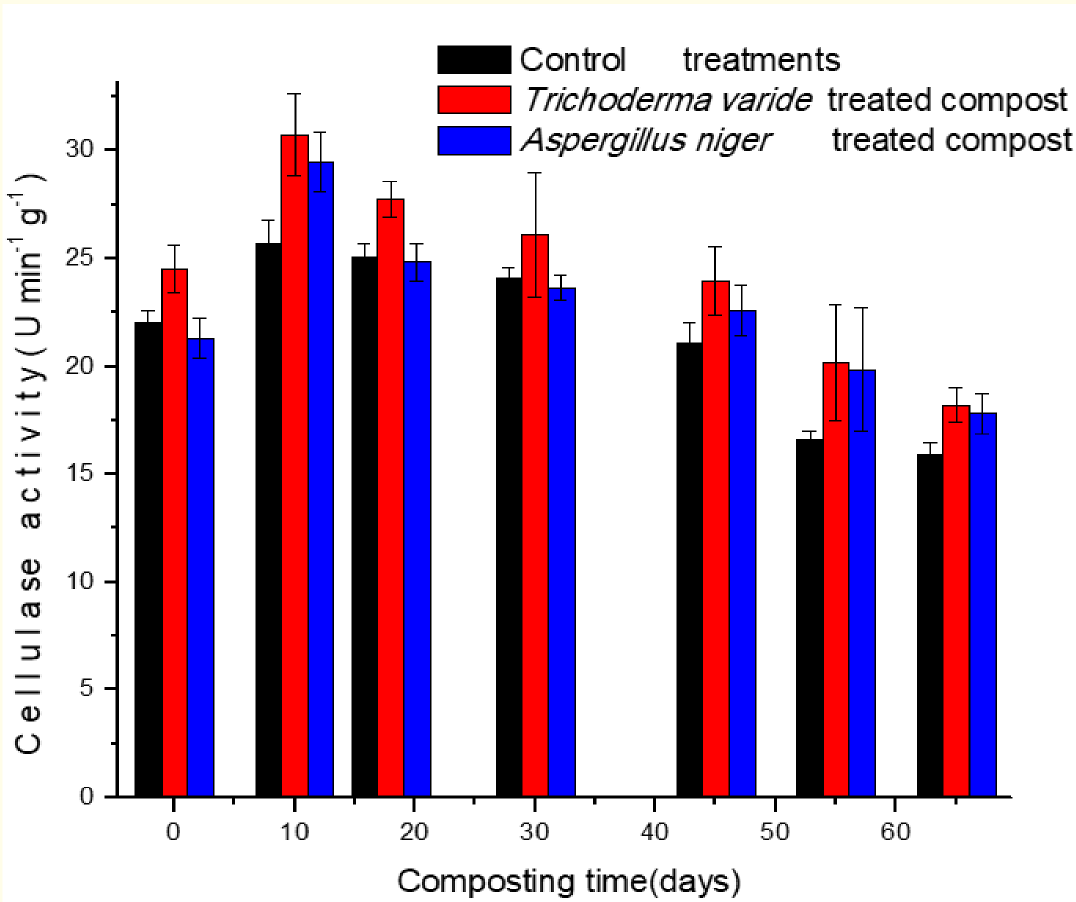


***Figure 14:*** *Cellulase activity dynamics during composting period.*

### Total metals

Metals are very crucial in agriculture for plant growth and developments but their high level are toxic to plants and soil microbes as well. Therefore, should be carefully monitored during quality compost production. Mineral nutrients (total metals) for matured compost are presented in table 1. Mineral nutrients such as Zn, Pb, Cu and Cr were very low compared to mineral limit established for compost by European guidelines [37]. Based on environmental quality classes for compost by European legislation, we found class 1 type compost form co-composting of digestate with saw dust and cow dung. The result macro nutrients (Na, K, Ca, Mg) were lower than result reported by [38] for compost obtained from pig slurry digestate.

| | | Compost | | | Mixed raw materials for compost |
|---|---|---|---|---|---|
| | Treatments | | | | |
| | Control treatments | Trichoderma viride treated | Aspergillus niger treated | DS+CD+SD | |
| ppm | Na | 1338 ± 127.0 | 1221.7 ± 12.0 | 1412 ± 146.0 | 1516.2 ± 45.0 |
| | K | 1620 ± 16.0 | 1027.5 ± 32.0 | 929 ± 154.0 | 2362.53 ± 121.0 |
| | Ca | 32840 ± 17.0 | 28690 ± 12.0 | 32223 ± 2700.0 | 37990.0 ± 178.0 |
| | Mg | 1315 ± 14.0 | 817.6 ± 168.0 | 1252 ± 352.0 | 1846.1 ± 244.0 |
| | Pb | 8.18 ± 3.1 | 8.9 ± 1.40 | 7.1 ± 1.90 | 9.12 ± 2.10 |
| | Cr | 0.14 ± 0.08 | 0.15 ± 0.14 | 0.14 ± 0.13 | 0.17 ± 0.50 |
| | Fe | 65 ± 3.0 | 30 ± 6.0 | 60 ± 10 | 74 ± 90.0 |
| | Zn | 0.77 ± 0.1 | 0.49 ± 0.01 | 0.84 ± 0.20 | 0.8 ± 0.10 |
| | Cu | 0.06 ± 0.01 | 0.04 ± 0.01 | 0.16 ± 0.03 | 0.14 ± 0.02 |
| | Mn | 22 ± 7.0 | 0.4 ± 0.10 | 5.0 ± 0.20 | 11.0 ± 2.0 |

***Table 1:** Mineral nutrients recorded for matured compost and raw materials used.*

*DS: Digestate; CD: Cow Dung; SD: Saw Dust and Values reported as mean ± standard error.*

**Germination Index (GI)**

In addition to physicochemical properties compost maturity and stability additionally can be supported using biological parameters like seed germination, root length and germination index [30,39]. Composts from all treatments showed absence of phytotoxins to seed of *Glycine max* germination after dilution of matured compost to 25%, 50%, 75% and 100%. Whereas raw digestate application showed strong toxicity to seed of *Glycine max* evidenced from GI% less than 50 (Table 2).

| Compost Percent dilution | Control/without fungal treatment | Compost treated with Trichoderma viride | Compost treated with Aspergillus niger | Raw digestate |
|---|---|---|---|---|
| 25 | 119.45 ± 0.34 | 90.34 ± 0.07 | 92.31 ± 0.15 | 50.15 ± 0.07 |
| 50 | 131.75 ± 0.18 | 86.50 ± 0.0 | 81.34 ± 0.08 | 53.75 ± 0.07 |
| 75 | 55.44 ± 0.06 | 79.15 ± 0.07 | 104.55 ± 0.32 | 25.1875 ± 0.0 |
| 100 | 63.14 ± 0.07 | 90.66 ± 0.07 | 55.50 ± 0.14 | 31.25 ± 0.07 |

***Table 2:** Compost germination index (GI, %).*

Therefore in we found, unsafe to use directly biogas digestate in agricultural practices due to its toxicity to seed germination. It may also toxic to soil biota if digestate used directly. According to [40] the proposed maturity indices for compost using germination index (GI) should be greater than 50%.

## Conclusions

Digestate obtained from anaerobic co-digestion of tannery wastes after biogas production contains valuable nutrients (micro and macro nutrients) which can be a great a fertilizer potential for agriculture use. But often difficulty to use directly digestate from tannery biogas in agricultural field due to its toxicity to seeds. From the present study we found high pH value in raw digestate. Raw digestate showed high toxicity to seed of *Glycine max* indicating of limiting factors for direct use of the digestate in crop production. There for, co-composting of digestate with saw dust and cow duding is crucial to get stable compost quality. After co-composting we found significant reduction in pH and no toxicity to seed germination compared to untreated digestate. Therefore, co-composting of the solid fraction of biogas digestate with cow dung and saw dust is one of the good option to obtained stable compost which can be used as fertilizer in put in agricultural.

## Acknowledgements

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