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# Catalase and peroxidase enzyme activities in pulp wood tree seedlings under textile sludge applied sandy loam and clay loam soils

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**Key words:** Textile sludge, pulp wood trees, catalase, peroxidase, sandy loam and clay loam soil.

**Abstract**

A pot culture experiment was carried out to study and compare the effect of terry towel textile sludge application on enzyme activity of fast growing pulp wood tree species raised under sandy loam and clay loam soils. The experiment was laid out in a Factorial Completely Randomized Block Design with two soil types *viz*., sandy loam soil ($C_1$) and clay loam soil ($C_2$); three fast growing pulp wood tree species viz., She Oak (*Casuarina junghuhniana* Miq.), The Forest Red Gum (*Eucalyptus tereticornis* Sm.) and The White Lead tree (*Leucaena leucocephala* Lam. de Wit). and six treatments having various dosage of terry towel textile sludge and Farm Yard Manure (FYM) including control viz., Control (without sludge and FYM), $T_1$ (12 t $ha^{-1}$ FYM - recommended dose), $T_2$ (23.8 t $ha^{-1}$ sludge - recommended dose), $T_3$ (12 t $ha^{-1}$ FYM + 23.8 t $ha^{-1}$ sludge - recommended dose), $T_4$ (47.6 t $ha^{-1}$ sludge - higher dose) and T5 (11.9 t $ha^{-1}$ sludge - lower dose). The activities of plant enzymes such as catalase and peroxidase were analyzed at 60 and 120 Days after Planting (DAP). Among the sludge and FYM treatments, application of 12 t $ha^{-1}$ FYM + 23.8 t $ha^{-1}$ sludge - recommended dose ($T_3$) exhibited significant effect on plant enzymes with 32.91 µg of $H_2O_2$ $g^{-1}$ $min^{-1}$ and 1.80 $g^{-1}$ $h^{-1}$ of catalase and peroxidase activity. The comparisons among three species revealed that *Eucalyptus tereticornis* ($S_2$) responded well in sludge treated soils and recorded significantly higher catalase activity; while *Casuarina junghuhniana* ($S_1$) exhibited higher peroxidase activity. The plant enzyme like catalase activity was significantly higher (23.18 µg of $H_2O_2$ $g^{-1}$ $min^{-1}$) in plants under sandy loam soil. However, peroxidase enzyme activity recorded higher (1.51 $g^{-1}$ $h^{-1}$) in plants under clay loam soil.

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**Introduction**

The textile industry is one of the oldest and largest sectors in India and it is amongst the top foreign exchange earning industries for the country. The textile units are scattered all over India; out of 21,076 units, Tamil Nadu alone has 5285 units (Bal, 1999). According to records of the Tamil Nadu State Pollution Control Board (TNPCB), there are 830 units engaged in textile industry processes in Tiruppur. Generally, textile mills produce mixed wastewater in large quantities up to 600 $m^3$ per kg fabric that are characterized by high organic load, up to 1000 mg COD/l. The wastewater treatment step concentrates the various pollutants in the wastewater into sludge, normally containing between 1 to 2 per cent by weight as dry solids (Priestly, 1991) and about 200 tons/day of textile sludge are generated in Tiruppur (Palanivelu and Rajakumar, 2001).

Textile sludge is a residual, semi-solid material left from industrial or waste water treatment processes. Metals that are associated with dye include Cu, Co, Cr, Ni and Zn. In addition, textile wastes contain high concentrations of some chloro - organics, solvents and phosphates that may limit their use for land treatment (Morris *et al.*, 1997, Balan and Monteiro, 2001).The inorganic salts and toxic metals in the sludge pose a threat to residents (Thomson, 1996, Palanivelu and Rajakumar, 2001, Islam *et al.,* 2009). This sludge creates more negative impacts in many ways if the correct disposal techniques are not adopted. Hence, the sludge produced is classified as hazardous, as per category No.12 of earlier hazardous waste, 1989 rules of India. The amended rules of January 2000 also classify the sludge as hazardous. However, Textile sludge obtained from waste water treatment has been classified as Non dangerous waste by European Waste Codes (MuraliKrishnan and Giridev, 2010).

Due to this controversy, the industry in India is under pressure to find a sustainable and cost-effective alternative to the safe disposal of textile industrial sludge. Processing and disposal of sludge that is economical and environmental friendly is currently of great importance to local authorities and industry. The disposal or reuse of sludge is a significant part of wastewater treatment programme. Disposing sludge is not guaranteed to be without any difficulty because the disposal options required at a time may not be feasible, that is why there is increasing agitation for sludge use or application. Often treated sludge is a resource that can be beneficially used in more than one way. Such use options might include direct application to forest plantation or making sludge compost and use them as slow release organic fertilizers for agricultural production (Morris *et al.*, 1997). At this moment, it is necessary to change the image of sludge and sludge treatment, from a “troublesome” into a “valuable” product. One way of helping to achieve this is to view sludge as by-product of wastewater treatment rather than as a waste product of textile industry. Selecting the best utilization or disposal options requires analysing the sludge end products for their characterization as well as their impact on the soil, plant and environment as a whole.

On the other hand, soils of waste land in India is low in organic matter content and  the decrease in soil organic matter is paralleled by declines in soil fertility, as many authors have demonstrated (Clapp *et al.*, 1986, Tate, 1987). One method to reverse this degradation in soil quality is the addition of organic matter (Bastian and Ryan, 1986). In recent years, composted urban waste has been added to agricultural land for both waste disposal and to improve soil fertility. Compost is rich in organic matter and an important source of nutrients for plants (Gallardo-Lara and Nogales, 1987) even though it may increase the level of potentially harmful trace metals and various persistent organic toxins (Giusquiani *et al.*, 1995). Studies of enzyme activities provide information on the biochemical processes occurring in the soil and there is growing evidence that soil biological parameters may have a potential as early and sensitive indicators of soil ecological stress and restoration (Dick and Tabatabai, 1992).

The aim of the present research was to study the possibility of the utilizing organic matter containing terry towel industrial sludge for forestry species especially to increase production of paper and pulp wood species by evaluating the changes in plant enzyme activity that took place in a pot culture soil that has been amended with textile sludge and FYM at different rates for important fast growing pulp wood tree species in India such as *Casuarina junghauhniana, Eucalyptus tereticornis* and *Leucaena leucocephala*. The specific plant enzymes activities such as catalase and peroxidase were measured.

## Materials

*Seed collection*

The seeds were collected from well grown, disease free and middle aged trees of *Casuarina junghauhniana, Eucalyptus tereticornis* and *Leucaena leucocephala* found in Forest College and Research Institute, Mettupalayam. India. The mature cones and pods were collected, shade dried properly and seeds were extracted carefully. The seeds were graded; Small sized, immature and malformed seeds were discarded.

*Collection and processing of textile sludge, FYM, sandy loam soil and clay loam soil*

The textile sludge was obtained from M/s. Sharada Terry Towel (textile) industry at Mettupalayam, Coimbatore district, Tamil Nadu state, India. The sludge (6 years old) was collected from the sludge dumped place with 50% moisture content. The soils used in this study are sandy loam of Irugur soil series and clay loam of Palathurai soil series. The Farm Yard Manure was collected from farm station of Tamil Nadu Agricultural University, Coimbatore. These samples were shade dried and sieved through 2 mm sieve and the processed samples were stored in polyethylene bags for further chemical analysis.

*Textile sludge generation*

Textile sludge is generated during the treatment of effluent of the industry. The solid wastes generated from treatment plants are brown in colour. The generation of sludge in M/s. Sharada Terry Towel (textile) industry can be depicted by the Fig. 1.

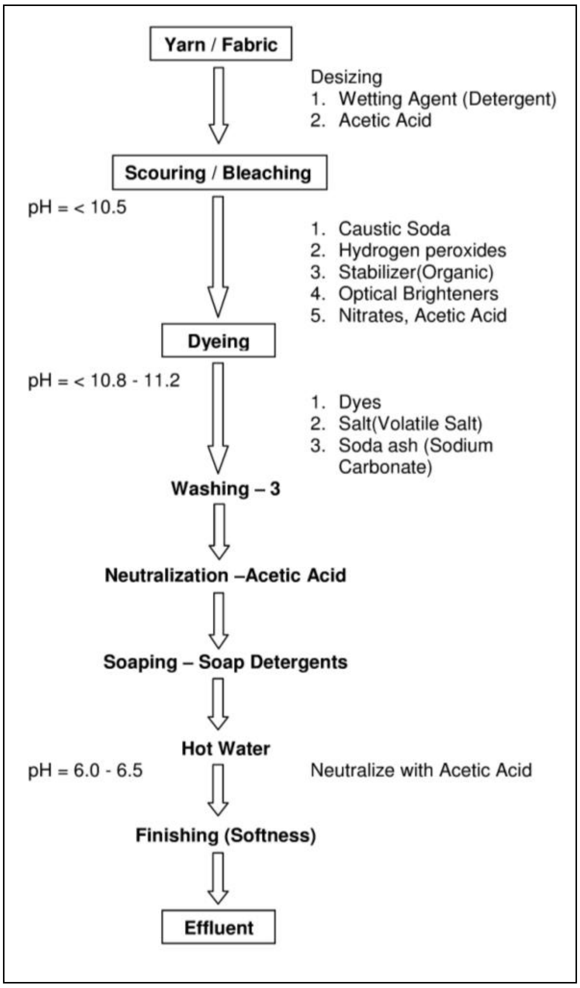


**Fig. 1.** The generation of sludge in M/s. Sharada Terry Towel (textile) industry

Textile raw effluent from the processing is treated in the Effluent Treatment Plant (ETP). Effluent is screened for removing the larger particulate matter and lime is dosed in the range of 200 ppm – 300 ppm and the envirofloc is dosed in the range of 1000 ppm - 1200 ppm for the formation of the flocs. Finally coagulating agent, polyelectrolyte is dosed at 10 ppm for formation of the larger flocs which is settable.

The sludge get settled at the bottom of the sedimentation tank is collected in the filter press feed tank. In the filter press sludge is collected in the cake form and the water is separated. Sludge collected is stored in the sludge storage yard. Sludge yard is built by providing the High Density Polyethylene (HDPE) lining on all sides and the leachate is connected to the raw water trench to the ETP.

**Methods**

*Physico-chemical properties of samples*

The methods used for the analysis of important Physico-chemical and biological properties of sludge, FYM and the experimental soil are presented in the Table 1 and Table 2.

**Table 1**. List of methods used for various physico-chemical properties of the samples used in the experiments

| Sl.No. | Properties | Method | Reference |
|---|---|---|---|
| 1. | pH | pH meter | Jackson (1973) |
| 2. | EC | Conductivity meter | Jackson (1973) |
| 3. | Organic Carbon | Wet digestion | Walkley and Black (1934) |
| 4. | Total Nitrogen | Kjeldahl apparatus | Bremner (1965) |
| 5 | Water holding capacity | Keen rackzkowski | Piper (1966) |
| 6. | Available Nitrogen | Alkali permanganate | Subbiah and Asija (1956) |
| 7. | Available Phosphorus | Colorimeter | Olsen *et al.,* (1954) |
| 8. | Available Potassium | Flame photometer | Jackson (1973) |

**Table 2**. Physical, chemical and biological characteristics of textile sludge, FYM and soils used in the experiment

| Sl.No | Parameters and units | Sludge | FYM | Sandy loam soil | Clay loam soil |
|---|---|---|---|---|---|
| 1. | pH[a] | 9.11 | 7.58 | 6.54 | 8.52 |
| 2. | Electrical conductivity ($dSm^{-1}$) | 0.51 | 2.70 | 0.09 | 0.20 |
| 3. | Moisture content (%) | 50 | 48.96 | ND[b] | ND[b] |
| 4. | Field capacity (%) | ND[b] | ND[b] | 17.83 | 35.56 |
| 5. | Permanent wilting point (%) | ND[b] | ND[b] | 8.07 | 25.12 |
| 4. | Water holding capacity (%) | 66.56 | 67.61 | 39.60 | 42.26 |
| 5. | Organic carbon (%) | 1.10 | 3.70 | 0.46 | 0.55 |
| 6. | Available nitrogen (%) | 0.02 | 1.20 | 0.02 | 0.01 |
| 7. | Available phosphorous (%) | 0.04 | 0.48 | 0.03 | 0.02 |
| 8. | Available potassium (%) | 0.18 | 0.52 | 0.02 | 0.01 |
| 9. | **Heavy metals ($mg\ kg^{-1}$)** | | | | |
| | Chromium (Cr) | 1.23 | 1.78 | 1.80 | 1.68 |
| | Cadmium (Cd) | 0.89 | 0.63 | 1.03 | 0.76 |
| | Lead (Pb) | 8.56 | 0.72 | 1.12 | 0.54 |
| | Nickel (Ni) | 12.68 | 1.05 | 1.44 | 1.15 |
| 10. | **Microbial load ($CFU\ g^{-1}$)** | | | | |
| | Bacteria | 126 | 192.23 | 16.89 | 12.67 |
| | Fungi | 41 | 54.27 | 5.33 | 5.33 |
| | Actinomycetes | 181 | 265.48 | 71.56 | 25.67 |
| 11. | **Soil enzymes** | | | | |
| | Phosphatase (µg PNPP $g^{-1}$) | 246 | 162 | 8.47 | 6.46 |
| | Dehydrogenase (mg TPF $g^{-1}$) | 235 | 154 | 7.59 | 5.29 |

[a] pH. Extractable soil water ratio for sludge 1:5, FYM, red and clay loam soil 1:2.5. , [b] ND (Not Determined).

*pH*

The pH of the sludge (1:5 – sludge: water mixture) was measured potentiometrically against reference electrode which is usually a calomel electrode. The potential difference between glass electrodes was expressed in pH units (Avudainayagam *et al.*, 2003).

*EC*

The electrical conductivity of the (1:5) sludge and water suspension was measured in terms of the resistance offered to the flow of current using a conductivity bridge (Avudainayagam *et al.*, 2003).

*Analysis of total metal content in sludge, soil and FYM*

The samples were digested with aqua-regia (3:1, HCl:$HNO_3$) and volume made up to 25 ml by using distilled water and the total metal (Cr, Pb, Ni, Cd) content were measured following standard instrument conditions using Varian Spectra AA 100/ 200 FAAS (Lindsay and Norvell, 1978).

*Optimization of textile sludge*

Based on the characterization of sludge the dose of sludge was optimized to conduct pot culture experiment in the sandy loam and clay loam soils. The dose of sludge was equated with the dose of FYM currently advocated to farmers. The recommended dose of FYM is 12.5 t/ha with general nitrogen content of 1.2 per cent. The sludge had a nitrogen content of 0.02 % and the dose was fixed at 24.8 t/ha.

*Analysis of enzyme activities*

**Catalase enzyme activity :** Leaf samples of 250 mg each were homogenized in 0.066 M phosphate buffer solution (pH 6.8) and centrifuged at 2000 rpm for 10 minutes. The reaction was activated by addition of 5 ml of phosphate buffer (pH 6.8) and 4 ml of 0.3 N hydrogen peroxide (substrate) to 0.2 ml of enzyme extract and was stopped after 15 minutes of incubation, by addition of 10 ml of 2 N $H_2SO_4$. The blank was maintained for each set with addition of 2 N $H_2SO_4$ in which 0.2 ml of distilled water was added. The samples were titrated against 0.1 N $KMnO_4$ and titrate values were noted down based on the appearance of pink colour. Difference between titrate values gave the volume of permanganate equivalent to enzyme activity (Povolotskaya and Sadenka, 1956).

**Peroxidase enzyme activity:** Leaf samples of 250 mg each were homogenized in 5 ml of 0.25 M Tris buffer (pH 6.0) and centrifuged at 5000 rpm for 10 minutes. The reaction mixture was prepared by addition of 0.5 ml of 1 per cent $H_2O_2$ and 0.5 ml of 0.5 per cent aqueous solution of pyrogallol to 0.4 ml of enzyme extract and incubated for 10 minutes at 25°C. The reaction was stopped by addition of 0.5 ml of 5 per cent $H_2SO_4$. The OD values at zero time and at the expiry of 10 minutes were measured in UV spectrophotometer at 420 nm. The peroxidase activity was calculated and the mean value was expressed as difference in OD $g^{-1}$ $hour^{-1}$ (Malik and Singh, 1980).

**Statistical Design:** The data were analyzed for the possible relationship between the different parameters and analysis of variance employing Factorial Randomized Block Design as described by Panse and Sukhatme, 1985 and data were analyzed by using AGRES software.

## Results

*Catalase enzyme activity*

Relatively significant variations were observed in catalase activity by the application of various combinations of textile sludge and FYM. Among them, seedlings treated with 12 t $ha^{-1}$ FYM + 23.8 t $ha^{-1}$ sludge - recommended dose ($T_3$) showed significantly higher catalase activity of 32.91 µg of $H_2O_2$ $g^{-1}$ $min^{-1}$ followed by $T_4$ (47.6 t $ha^{-1}$ sludge - higher dose), $T_2$ (23.8 t $ha^{-1}$ sludge – recommended dose), $T_1$ (12 t $ha^{-1}$ FYM), $T_5$ – 11.9 t $ha^{-1}$ sludge (lower dose) and control with recorded values of 26.55, 23.53, 17.46, 15.53 and 12.53 µg of $H_2O_2$ $g^{-1}$ $min^{-1}$ respectively.

Between the two soil types, plants under sandy loam soil recorded higher catalase activity of 23.18 µg of $H_2O_2$ $g^{-1}$ $min^{-1}$ over clay loam soil which recorded a value of 19.66 µg of $H_2O_2$ $g^{-1}$ $min^{-1}$. In sandy loam soil, *Eucalyptus tereticarnis* ($S_2$) recorded higher (28.79 µg of $H_2O_2$ $g^{-1}$ $min^{-1}$) catalase activity. The least catalase activity was recorded in *Leucaena leucocephala* ($S_3$) (17.64 µg of $H_2O_2$ $g^{-1}$ $min^{-1}$). Same trend was observed in clay loam soil where, *Eucalyptus tereticornis* ($S_2$) ranked first position with 23.67 µg of $H_2O_2$ $g^{-1}$ $min^{-1}$ and the least value was recorded in *Leucaena leucocephala* ($S_3$) with 17.26 µg of $H_2O_2$ $g^{-1}$ $min^{-1}$ (Table 3).

**Table 3.** Catalase activity (µg of $H_2O_2$ $g^{-1}$ $min^{-1}$) influenced by soil types, textile sludge and tree species at 90 and 180 DAP.

| Soil type (C) | Sludge Doses (T) t $ha^{-1}$ | Pulp wood tree species (S) | | | | | |
|---|---|---|---|---|---|---|---|
| | | *Casuarina junghuhniana* ($S_1$) | | *Eucalyptus tereticornis* ($S_2$) | | *Leucaena leucocephala* ($S_3$) | |
| | | P1 | P2 | P1 | P2 | P1 | P2 |
| **Sandy loam ($C_1$)** | **Control** | 12.45 ± 0.59 | 13.16 ± 008 | 18.35 ± 0.24 | 20.42 ± 0.03 | 10.03 ± 0.57 | 12.20 ± 0.08 |
| | **$T_1$** | 25.14 ± 0.64 | 26.85 ± 0.58 | 29.38 ± 0.16 | 30.23 ± 0.28 | 17.33 ± 0.04 | 18.35 ± 0.11 |
| | **$T_2$** | 20.10 ± 0.57 | 21.25 ± 0.58 | 24.36 ± 0.36 | 26.26 ± 0.01 | 14.25 ± 0.14 | 16.24 ± 0.58 |
| | **$T_3$** | 32.18 ± 0.03 | 32.47 ± 0.87 | 42.95 ± 0.14 | 46.32 ± 0.69 | 28.20 ± 0.17 | 30.13 ± 0.08 |
| | **$T_4$** | 27.53 ± 0.04 | 28.36 ± 0.02 | 31.25 ± 0.06 | 34.22 ± 0.14 | 18.26 ± 0.16 | 19.25 ± 0.06 |
| | **$T_5$** | 18.17 ± 0.08 | 19.49 ± 0.97 | 18.35 ± 0.19 | 20.42 ± 058 | 13.21 ± 0.12 | 14.17 ± 0.09 |
| **Clay loam ($C_2$)** | **Control** | 8.53 ± 0.03 | 9.50 ± 0.29 | 17.20 ± 0.15 | 17.85 ± 0.46 | 05.68 ± 0.04 | 05.00 ± 0.13 |
| | **$T_1$** | 15.37 ± 0.12 | 16.21 ± 0.11 | 25.43 ± 0.09 | 26.35 ± 0.57 | 25.33 ± 0.19 | 26.33 ± 0.57 |
| | **$T_2$** | 14.20 ± 0.06 | 15.27 ± 0.24 | 19.75 ± 0.55 | 17.73 ± 0.09 | 10.63 ± 0.01 | 9.52 ± 0.30 |
| | **$T_3$** | 28.25 ± 0.10 | 30.50 ± 0.06 | 31.20 ± 0.17 | 35.23 ± 0.59 | 28.15 ± 0.15 | 29.34 ± 0.58 |
| | **$T_4$** | 25.27 ± 0.09 | 26.40 ± 0.55 | 28.37 ± 0.04 | 29.43 ± 0.22 | 25.23 ± 0.14 | 25.01 ± 0.34 |
| | **$T_5$** | 14.37 ± 0.31 | 12.88 ±0.16 | 17.20 ± 0.15 | 17.85 ± 0.19 | 8.70 ± 0.56 | 8.17 ± 0.06 |
| ANOVA | C | P = 0.055** | | P | P = 0.055** | | |
| | T | P = 0.095** | | C X S X T X R | P = 0.331** | | |
| | S | P = 0.067** | | | | | |

Values are represented in mean ± Standard Error with three replicates, P = Test of significance, **Indicates significance at 0.01%, ANOVA-Analysis of Variance, T-Treatment, S-Species. P1 – 90 DAP; P2 – 180 DAP; Control (Without sludge and FYM), $T_1$ (12 t $ha^{-1}$ FYM – recommended dose), $T_2$ (23.8 t $ha^{-1}$ sludge - recommended dose), $T_3$ (12 t $ha^{-1}$ FYM + 23.8 t $ha^{-1}$ sludge - recommended dose), $T_4$ (47.6 t $ha^{-1}$ sludge - higher dose) & $T_5$ (11.9 t $ha^{-1}$ sludge - lower dose).

*Peroxidase enzyme activity*

Among the treatments, $T_3$ depicted maximum peroxidase activity of 1.80 $g^{-1}$ $h^{-1}$ followed by $T_3$, $T_2$, $T_1$, $T_5$ and control with a recorded values of 1.43 $g^{-1}$ $h^{-1}$, 1.32 $g^{-1}$ $h^{-1}$, 0.70 $g^{-1}$ $h^{-1}$, 0.57 $g^{-1}$ $h^{-1}$ and 0.49 $g^{-1}$ $h^{-1}$ respectively. Between two soil types, plants under clay loam soil recorded maximum peroxidase activity of 1.51 $g^{-1}$ $h^{-1}$ while minimum (0.59 $g^{-1}$ $h^{-1}$) was observed in sandy loam soil.

Relatively significant variations were observed in peroxidase activity due to the influence of type of soil and tree seedlings. In sandy loam soil, *Casuarina junghuhniana* recorded significantly higher (0.65 $g^{-1}$ $h^{-1}$) peroxidase activity. The least recorded was in *Eucalyptus tereticarnis* (0.55 $g^{-1}$ $h^{-1}$). In clay loam soil *Casuarina junghuhniana* retains its first position with 1.93 $g^{-1}$ $h^{-1}$ followed by *Eucalyptus tereticarnis* (1.75 $g^{-1}$ $h^{-1}$) and *Leucaena leucocephala* (0.86 $g^{-1}$ $h^{-1}$). Within the three species, *Casuarina junghuhniana* depicted maximum peroxidase activity of 1.29 $g^{-1}$ $h^{-1}$ which was followed by *Eucalyptus tereticarnis* and *Leucaena leucocephala* with a recorded value 1.15 $g^{-1}$ $h^{-1}$ and 0.71 $g^{-1}$ $h^{-1}$ respectively. However, the rate of increase in peroxidase enzyme from 90 DAP – 180 DAP was higher in *Eucalyptus tereticarnis* with 194% higher than control when compared to *Casuarina junghuhniana* and *Leucaena leucocephala* which were 129 % and 93 % respectively more over control (Table 4).

**Table 4.** Peroxidase activity ($g^{-1}$ $h^{-1}$) influenced by soil types, textile sludge and tree species at 90 and 180 DAP.

| Soil type (C) | Sludge Doses (T) t $ha^{-1}$ | Pulp wood tree species (S) | | | | | |
|---|---|---|---|---|---|---|---|
| | | *Casuarina junghuhniana* ($S_1$) | | *Eucalyptus tereticornis* ($S_2$) | | *Leucaena leucocephala* ($S_3$) | |
| | | P1 | P2 | P1 | P2 | P1 | P2 |
| Sandy loam ($C_1$) | Control | 0.42 ± 0.006 | 0.47 ± 0.012 | 0.19 ± 0.006 | 0.23 ± 0.012 | 0.13 ± 0.009 | 0.17 ± 0.006 |
| | $T_1$ | 0.40 ± 0.007 | 0.42 ± 0.003 | 0.34 ± 0.012 | 0.33 ± 0.006 | 0.45 ± 0.012 | 0.53 ± 0.021 |
| | $T_2$ | 0.48 ± 0.003 | 0.53 ± 0.009 | 0.27 ± 0.003 | 0.34 ± 0.009 | 0.17 ± 0.009 | 0.27 ± 0.006 |
| | $T_3$ | 0.83 ± 0.006 | 0.80 ± 0.006 | 0.77 ± 0.009 | 1.26 ± 0.015 | 0.94 ± 0.015 | 1.07 ± 0.010 |
| | $T_4$ | 1.43 ± 0.021 | 1.13 ± 0.028 | 1.06 ± 0.015 | 1.36 ± 0.010 | 1.14 ± 0.015 | 1.22 ± 0.009 |
| | $T_5$ | 0.46 ± 0.003 | 0.44 ± 0.003 | 0.23 ± 0.006 | 0.26 ± 0.012 | 0.33 ± 0.006 | 0.38 ± 0.003 |
| Clay loam ($C_2$) | Control | 0.78 ± 0.003 | 0.82 ± 0.012 | 0.65 ± 0.009 | 0.70 ± 0.007 | 0.08 ± 0.003 | 1.22 ± 0.006 |
| | $T_1$ | 2.44 ± 0.009 | 2.54 ± 0.015 | 2.26 ± 0.024 | 2.65 ± 0.007 | 1.65 ± 0.012 | 1.86 ± 0.015 |
| | $T_2$ | 1.54 ± 0.013 | 1.65 ± 0.007 | 1.35 ± 0.015 | 1.67 ± 0.013 | 0.08 ± 0.003 | 0.06 ± 0.003 |
| | $T_3$ | 2.87 ± 0.006 | 2.95 ± 0.006 | 2.44 ± 0.009 | 2.95 ± 0.025 | 2.50 ± 0.027 | 2.23 ± 0.007 |
| | $T_4$ | 2.37 ± 0.012 | 2.66 ± 0.015 | 2.06 ± 0.033 | 2.15 ± 0.010 | 0.04 ± 0.003 | 0.54 ± 0.012 |
| | $T_5$ | 1.27 ± 0.009 | 1.31 ± 0.007 | 1.01 ± 0.003 | 1.08 ± 0.003 | 0.03 ± 0.003 | 0.01 ± 0.003 |
| ANOVA | C | P = 0.007** | | | P | P = 0.007** | |
| | T | P = 0.012** | | | C X S X T X R | P = 0.043** | |
| | S | P = 0.009** | | | | | |

Values are represented in mean ± Standard Error with three replicates, P = Test of significance, **Indicates significance at 0.01%, ANOVA - Analysis of Variance, T-Treatment, S-Species. P1 – 90 DAP; P2 – 180 DAP; Control (Without sludge and FYM), $T_1$ (12 t $ha^{-1}$ FYM – recommended dose), $T_2$ (23.8 t $ha^{-1}$ sludge - recommended dose), $T_3$ (12 t $ha^{-1}$ FYM + 23.8 t $ha^{-1}$ sludge - recommended dose), $T_4$ (47.6 t $ha^{-1}$ sludge - higher dose) & $T_5$ (11.9 t $ha^{-1}$ sludge - lower dose).

**Discussion and conclusion**

In the present study, addition of textile sludge to pot culture soil significantly enhanced the catalase and Peroxidase activity compared to control. This may probably because of the fact that textile sludge has a high amount of utilizable substrates for microbial growth. Similar result was observed by Ros *et al.,* 2003 in sewage sludge. According to our experiments, plants raised in soils treated with textile sludge and FYM at recommended dose significantly increased catalase and peroxidase activity, thus suggesting the important role of FYM in the sludge decomposition and release of nutrients for plant growth. Dehydrogenase and catalase are intracellular enzymes that are involved in microbial oxidoreductase metabolism (Rodriguez-Kabana and Truelove, 1982) and its activity was significantly and positively correlated with microbial biomass and b-glucosidase in the accumulative plots (García-Gil *et al.,* 2000). This result may be explained by the improved soil aeration in the organic amended soils as a consequence of an increase in soil porosity (Giusquiani *et al*., 1995). The activity of such enzymes basically depends on the metabolic state of the soil biota and dehydrogenase activity could be a good indicator of soil microbial activity in semiarid areas (Garcia *et al*., 1994). The increase in catalase and peroxidase activity in plants under soil applied with textile sludge and FYM presumably due to the humified organic matter added with textile sludge, which is more resistant to microbial mineralization. Incorporation of organic materials, such as FAM, into soil promotes microbiological activity. Microbial activity and soil fertility are generally closely related because it is through the biomass that the mineralization of the important organic elements (C, N, P and S) occur (Frankenberger and Dick, 1983). Other authors have reported that dehydrogenase activity was inhibited by the toxic effect of heavy metals added with an organic amendment, particularly Pb (Marzadori *et al*., 1996) and Cu (Chander and Brookes, 1991a), but the levels of these heavy metals in textile sludge (Table 2) were much lower than those reported by previous investigations and, in any case, this enzyme was not affected by the heavy metal concentrations reached in soil with the highest rate of textile sludge amendment.

The soil types affected the plant enzymes (catalase and peroxidase) with great significance. The catalase enzyme activity was significantly higher in plants under sandy loam soil than plants under clay loam soil. The increased uptake of moisture and nutrient by improved root penetration and increased availability of nutrients in sandy loam soil might have increased the catalase activity when compared to clay loam soil. However, the peroxidase enzyme activity was significantly higher in clay loam soil. The mechanism behind the increased peroxidase activity of plants in clay loam soil over of sandy loam soil was not clearly understood.

Significant variations were observed in catalase activity in the three tree species. Catalase activity was higher in *Eucalyptus tereticornis*. However, the rate of increase in catalase activity from 90 – 180 DAP was higher in *Leucaena leucocephala* with 134 % than control followed by *Casuarina junghuhniana* and *Eucalyptus tereticornis* they were 106 % and 50 % respectively higher over control. This may probably the root nodulation character of *Casuarina junghuhniana* and *Leucaena leucocephala* when compared to *Eucalyptus tereticornis* which is a non leguminous and non nodulation tree species.

Increased peroxidase activity in *Casuarina junghuhniana* may be due to its spreading, fibrous root system that can penetrate quite deeply into the soil and may have the chances of up-taking more moisture and nutrients in the pot culture soil. Moreover, the root hairs become infected by *Frankia spp*. and form nitrogen-fixing nodules (Mowry, 1933 and Torrey, 1976) which may also be the reason for higher peroxidase activity. However, the rate of increase in peroxidase enzyme was significantly higher in *Eucalyptus tereticornis* with 194 % over control when compared to *Casuarina junghuhniana* and *Leucaena leucocephala* which were 129 % and 93 % respectively more over control. This may be due to the reason that *Eucalyptus tereticornis* might have uptake more water, nutrients and heavy metals from the amended pot mixture soil and produced more peroxidase enzyme. This is in agreement with the statement that *Eucalyptus* has inherent capacity to draw more water and nutrients from soil and the fine root density of *Eucalyptus* is higher in upper soil which plays a crucial role in water uptake even during dry season (Bouillet *et al*., 2002). Similarly, Stape *et al*., 2004 concluded that water use efficiency of *Eucalyptus* species was higher i.e. 1.8 - 3.8 kg $m^{-3}$ compared to other tree species.

Overall, our results have shown that the addition of textile sludge and FYM to pot culture soil has a variety of effects on plant enzyme activities. Soils amended with textile sludge can improve soil quality by improving soil biological and biochemical properties which in turn helps in producing improved quality seedlings for establishing pulp wood plantation in India. However, the wide variety of substances such as heavy metals and other potential pollutants in textile solid waste limits the use of these residues as organic amendments. Consequently, there must be a quality control of these organic amendments in order to minimise the risk of inhibiting essential biogeochemical processes as well as contributing to environmental pollution. In the study, plant enzymes showed different behaviour in soil types (sandy loam and clay loam), different pulp wood tree seedlings as well as in different growth periods. Hence, long term study is required for understanding the response of pulp wood trees with special emphasis on plant enzyme activities in sandy loam and clay loam soils by the addition of textile sludge.

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