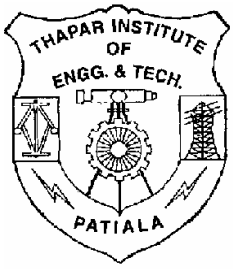
# Impact of fly ash on wood quality of *Eucalyptus tereticornis*

**A**

**DISSERTATION**

**Submitted in partial fulfillment of the requirements**
**for the Award of the Degree of**
**Masters of Science in Biotechnology**

**Under the Guidance of**
**Dr. Dinesh Goyal**
**Project Supervisor**

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# CERTIFICATE

This is to certify that the thesis by Jatinder Singh (Roll No. 3030105) in partial fulfilment of the requirements for the award of Degree of Master of Sciences in Biotechnology, to Thapar Institute of Engineering and Technology (Deemed university), Patiala, is a record of student's own work carried out by him under my supervision and guidance. The report has not been submitted for the award of any other degree or certificate in this or any other university or institute.

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# Abstract

*Eucalyptus* wood grown in the soil amended with (ESP) fly ash @ 0%, 6%, 12%, 18% and 24% v/v basis, involving different treatments was harvested from six year old plantation and studied for various structural polysaccharides cellulose, hemicellulose, lignin and heavy metals Cu, Cr, Fe, Ni, Pb.

Among various structural polysaccharides, cellulose in pulpable wood was in the range of 32.6-47.7%, hemicellulose was in the range of 8.5-10.2%, lignin was in the range of 29.7-40.6% and ash was in the range of 2.9-4.5%. Fly ash at 12% (v/v) addition in soil had no detrimental effect on any of these polysaccharide components of wood, rather caused an increase in cellulose content, whereas lignin content remained almost normal which was at par with wood grown in soils without fly ash. The ash and hemicellulose content in wood did not vary significantly. Heavy metals (Cu, Cr, Fe, Ni, and Pb) varied significantly in *Eucalyptus* wood grown in soil with different fly ash concentrations. Overall Cu ranged from 1.6-3.8 mg/Kg, Cr ranged from 5.6-17.6 mg/Kg, Fe ranged from 112.8-248.3 mg/Kg, Ni ranged from 1.3-15.3 mg/Kg and Pb ranged from 3.7-21.1 mg/Kg. With increased addition of fly ash at 18-24% level the uptake of metal by wood was increased**,** however at 12% fly ash these metals were more or less in the range at par with wood grown in soil without fly ash.

No correlation could be established between structural polysaccharides and heavy metal content of wood. 12% fly ash from ESP appears to be an optimum dose, which can be judiciously added to soil in order to improve yield and cellulose content of wood.

# Table of Contents

# 1. Introduction

Nearly 73% of India's total installed power generation capacity is thermal, of which coal-based generation is 90%, the remaining comprising diesel, wind, gas, and steam. The 85 utility thermal stations, beside several captive power plants, use bituminous and sub-bituminous coal and produce large quantities of fly ash. High ash content (30%-50%) contributes to large volume of fly ash. Even as we forge ahead into the new millennium, India's dependence on coal as a source of energy remains unchanged. Fly ash management and its proper disposal is an important source of national concern. With more thermal plants coming up, generation of fly ash is likely to increase up to 150 million tones per annum. Sites for disposal of this ash would consume around 30,000 hectares of land creating various types of environmental pollution.

Fly ash contains various types of heavy metals such as iron, cobalt, lead etc. In loose form, when inhaled it causes bronchial asthma. It also contaminates ground water. On the other hand, it also contains various types of micro and macronutrients for the plants and enhances the fertility of the soil. Due to these properties of the fly ash various types of economically important plants especially multipurpose tree species can be grown on it, which can be used as fuel and in pulp and papermaking etc.

*Eucalyptus* has emerged as a multipurpose tree species to be grown on waste degraded land due to its high adaptability and fast growth rate and among different species *E. tereticornis* has proved superior in its adaptation. It is a hardwood plant and can grow in a variety of soil conditions and is an important source of wood fiber. *Eucalyptus* is a fiber source upon which three industries- packaging materials, fine paper and newsprint could be built. There are a number of ways in which hardwood in general and *Eucalyptus* in particular may be regarded, in terms of pulping characteristics, as superior to softwood and vice-versa. *Eucalyptus* has lower lignin

content, higher hemicellulose and higher sensitivity to swelling agents therefore pulp has low flow resistance and shows rapid strength development on beating.

In paper and pulp industry, wood quality is an important parameter. Its properties determine its suitability for further processing. Structural features of plant fiber considered important are: average length, diameter, wall thickness and wall ultra structure. Fiber wall ultra structure is determined by cellulose, hemicellulose and lignin content. Cellulose is desired in high amount as it forms the skeleton of the plant fiber. High hemicellulose is desired as it is hydrophilic that helps in internal lubrication of fiber, improved flexibility, increased sheet density and ease of mechanical refining. Lignin is hydrophobic in nature and it decreases inter-fiber bonding in paper therefore removal of lignin is the main objective of the pulping and bleaching process.

Trees when grown in soils contaminated with industrial solids wastes may have altogether different fiber wall structure, which is reflected in varied composition of cellulose, hemicellulose and lignin, which may affect pulping characteristics. Similarly, if the waste admixed with soil contains heavy metals that may also lead to higher uptake of metal in plants, which finally in the harvested wood may result in different pulping characteristics/process. Therefore, in the present investigation the wood quality parameters such as cellulose, hemicellulose and lignin were determined in *Eucalyptus* wood harvested from six-year-old plantation grown in fly ash amended soils. Additionally the heavy metals in the wood were determined in order to assess if there is any effect of fly ash amendment in soil on heavy metal uptake.

# 2. Review of Literature

Up to 90% of the ash in many coal-fired plants is fly ash (Klein *et al*., 1975). Fly ash is primarily composed of silt-sized, glassy spheres with numerous impurities within and on the surface of these small silt and sand like particles. These spheres can be solid, hollow, irregularly shaped, or filled with smaller particles. Fly ash generally has a silt loam texture with 65-90% of the particles having diameter of less than 0.01mm (Chang *et al*., 1977; Roy *et al*., 1981). Ash from bituminous coal is usually finer than produced by the burning of lignite (Tolle *et al.,* 1982). Theis and Wirth (1977) found that the major component were Al, Fe and Si, with smaller concentrations of Ca, K, Na, Ti, and S.

In 1998, total U.S. fly ash production was 63 million tons (ACAA, 1999). Most of the fly ash presently produced by electric utilities and industry is land filled or stored in disposal pond, although approximately 33% was beneficially utilized for various purposes in 1998 (ACAA, 1999). The World Bank has cautioned India that by 2015, disposal of coal ash would require 1000 square kilometers or one square meter of land per person. Since coal currently accounts for 70% of power production in the country, the bank has highlighted the need for new and innovative methods for reducing impact on the environment. In its report, *Environment issues in the power sector*, the bank uses a decision-making tool to assess strategies for minimizing environmental impact of expanding power generation (*The Business Line, 22 December 1999*).

To prevent the fly ash from getting air borne, the dumping sites have to be constantly get wet by sprinkling water over the area. The coal industry in USA spends million of dollars on lining fly ash dumping grounds. But in India, these sites are not lined and lead to seepage, contaminating the ground water and soil. It lowers soil fertility and contaminates surface and ground water as it can leach into the subsoil. When fly ash gets into the natural draining system, it results into siltation and clogs the system. It also reduces the pH balance and portability of water. Fly ash interferes with the

process of photosynthesis of aquatic plants and thus disturbs the food chain. Besides, fly ash corrodes exposed metallic structure in its vicinity.

Fly ash is a serious source of air pollution since it remains air born for a long period and causes health problems. Fugitive dust and heavy metal contamination in the ground water are the major problem for the local masses. Swallowing of fly ash may cause abdominal discomfort. It causes irritation to the eyes (watering and redness), skin (irritant/contact dermatitis from mechanical abrasion or alkaline composition) and nose, and respiratory tract (coughing and sneezing). Repeated inhalation of fly ash dust containing silica can cause bronchitis, silicosis (scarring of lungs) and lung cancer. It may also increase the risk of scleroderma (a disease affecting the connective tissues the skin, joints, blood vessels and internal organs).

## Tackling the problem of fly ash

Fly ash management has taken considerable strides over the part few years. Researchers have been attempting to convert this waste into wealth by exploring viable avenues for fly ash management. Fly ash is oxide rich and can be used as the raw material for different industries. Fly has a great potential for use as a building material. The American Embassy in India has used fly ash bricks in some of its recent constructions. Use of fly ash as a partial replacement of cement in mortar and concrete has started with The Indian Institute of Technology, Delhi taking the lead. Use of fly ash for construction of roads has been successfully demonstrated and is gaining acceptance. NTPC (National Thermal Power Corporation) is setting up two fly ash brick manufacturing plants at Badarpur and Dadri near Delhi. Apart from using fly ash as a substitute for bricks, it can also be used for embanking road. Meanwhile the DMRC (Delhi Metro Rail Corporation) has adopted the technique for construction of railway embankments and land filling. The DMRC has adopted the technique for filling up land in the Shastri Park area located on Shahdara-Tis hazari sector in New Delhi, using 950,000 cubic meters of fly ash from the Indraprastha and Rajghat power station mixed with soil in a predetermined ratio. As the production of fly ash is increasing day by day, utilization of coal fly ash should be stimulated as

much as possible through implementation of new economically feasible and environmentally sound applications to safeguard the interest of human life, wild life and other such considerations.

## Use of fly ash for growing economically important plants

It has been demonstrated that fly ash can be used for the cultivation of economically important plants as it has many beneficial properties. It improves permeability status of soil, enhances root proliferation, improves soil texture and improves water-holding capacity of the soil and soil aeration. It also provides micronutrients like Fe, Zn, Cu, Mo, etc. and macronutrients like K, P, and Ca etc. The addition of appropriate quantities of fly ash can alter the soil texture. Fly ash addition @ 70t/ha has been reported to alter the soil texture of sandy and clayey soil to loam (Fail and Wochock, 1977). The application of fly ash has been found to increase the available water content of loamy sand soil by 120% and of sandy soil by 67% (PAU, Ludhiana). In India most of the fly ash produced is alkaline in nature. Hence an application of these to agricultural soil increases the soil pH. This property of fly ash can be exploited to neutralize acidic soils (Elseewi *et al*. 1978; Phung *et al.* 1978).

## Effect on growth and yield of crops

Regional Research Laboratory (RRL), Bhopal reported that on an average in comparison to control around 50-60% more yield of brinjal, around 45% more yield of potato and pea, around 40% more yield of tomato were recorded in fly ash treated plot when fly ash was applied @ 25% of soil. Punjab Agriculture University (PAU) observed that application of fly ash @ 10 t/ha increased the yield of wheat from 21.5 q/ha to 24.1 q/ha and that of cotton from 1245 kg/ha to 1443 kg/ha. At college of agriculture-Raichur yield of sunflower was increased by about 25% in red soil under rained as well as irrigated conditions when fly ash was applied @ 60t/ha along with 20t/ha FYM. More than 70% increase in yield of groundnut was observed when fly ash was applied @ 30 t/ha along with FYM @ 20 t/ha at CAS Raichur. TERI researchers have succeeded in cultivating a variety of economically important plants in a fly ash pond. Marigolds, tuberoses, carnations, sunflowers, and a variety of trees

including *Poplar* (*Populus deltoids*), '*Sheesham*' (*Dalbergia sisso*) and *Eucalyptus* are thriving on the mound of fly ash in Badarpur. A 3000- square meter abandoned fly ash pond on the Badarpur Thermal Power Station is the site of green miracle. A portion of the pond-dead down several meters with fly ash-is now reaping the harvest of the revolutionary environmental technology (*The Indian Express, 30 September 1999*). The vegetative cover on the site benefits the humans too. Ash from the dumping sites is bound together by the plant roots so hazard due to air borne fly ash is minimized and due to other toxic materials is also reduced.

## Cultivation of *Eucalyptus* in fly ash

In the recent years *Eucalyptus* has emerged as a preferred tree for reclamation of the fly ash dumping sites due to its various properties. The genus *Eucalyptus* belongs to the family Myrtaceae with about 3000 known species. It is fastest growing tree in the world and many species attain great heights and a native of Australia and Tasmania, was introduced in India, by the British in 1843 in Nilgiri Hills as an experiment to grow high yielding species for fuel and timber. It soon became a favoured species for the foresters /commercial plantation, owing to its fast growth, drought resistant nature and adaptability to a variety of agro climatic conditions. It is popularly known as red iron tree, nilgiri or safeda.

## Botanical features

*Eucalyptus* is a fast growing, medium-sized to tall tree attain 20-50m in height and up to 2m in diameter the tree has a deep tap root system which increases its ability to draw nutrients and water. The tree has a smooth silvery white stem. The leaves are leathery texture, hang obliquely or vertically and studded with glands containing glands containing aromatic oil. Flowering takes place during July-August. Flowers in the bud are covered with a cup like membrane (whence the name of the genus, derived from the Greek 'eucalyptus' meaning-'well covered'), which is thrown off as a lid when the flower expands. The fruiting occurs during September-October. The fruits are surrounded by a woody, cup- shaped receptacles and contains numerous minute seeds.

## Silviculture characteristics

*Eucalyptus* shows a wide range of soil and climatic adaptability. *E. tereticornis* has been planted in a wide climate range with mean annual temperature of 17-40°C. Basically a light demander, the growth of the species is very much reduced under the shade. *Eucalyptus* is known for its drought hardiness, although annual rainfall of 800 mm is preferred. The species is moderately salt tolerant and relatively fire resistant. *E. tereticornis* has known to come up with reasonable success in regions where unseasonable frost is likely to occur. *Eucalyptus* in India has been found susceptible to low temperature and snow causes damage to plantation. Water logged or sites with heavy soil and sandy soils are not suitable for raising *Eucalyptus* plantation. The *Eucalyptus* may be reduced in size under the harsh conditions (Tewari, 1992).

## Yield

Considerable work has been done on the growth behavior of *Eucalyptus* on different sites. Total biomass of 81.1 t/ha was obtained from 5-year-old *Eucalyptus tereticornis* grown in U.P. (Singh and Sharma, 1976). The yield figures for coppice growth for *Eucalyptus tereticornis* as reported by Ghosh *et al*. (1977) are given below.

**Volume ($m^3$/ha) of *E. terticornis* (coppice)**

| Age (yr.) | Diameter (cm.) | Volume ($m^3$/ha) | |
|---|---|---|---|
| | | Over bark | Under bark |
| 2 | 4.9 | 14.14 | - |
| 4 | 7.7 | 88.2 | 70.77 |
| 6 | 9.4 | 207.82 | 162.51 |
| 4 | 6.5 | 58.15 | 20.46 |
| 5 | 8.9 | 66.66 | 47.34 |

## Commercial uses of *Eucalyptus*

1. *Eucalyptus* is one of the fastest growing trees and is an excellent timber for paper and pulp and hardboard industries.
2. It is also an excellent source of fuel wood and charcoal.
3. *Eucalyptus* wood is also used for light and heavy constructions, railways sleepers, bridges, piles poles and mining timber.
4. Indian Standards are available for the use of *E. tereticornis* timber, after treatment, for doorframes, windows shutters, furniture, cabinet, tool handlers and crates.
5. Leaf extract of the *Eucalyptus* species has pesticide properties and can be promoted as biopesticide.
6. The leaves of the species are rich in essential oils that have many medicinal properties. *Eucalyptus globules* can be raised commercially for *Eucalyptus* oil.
7. The wood and the bark of the tree have a tannin content of 6-12% and 3-15% respectively.
8. *Eucalyptus* is a large ornamental tree suitable for the parks and avenue plantations.
9. The tree can be effectively used for regeneration of denuded lands and prevention of soil erosion in drought affected areas.
10. The tree may be used as an agro-forestry species. *Eucalyptus* in combination with pineapple has given excellent results in china.

## Hardwood vs. Softwood

Wood species can be broadly classified into two groups: (1) wood of coniferous tree is termed as 'softwood' and (2) wood of broad-leaved trees or deciduous trees (angiosperms) as 'hardwood'.

### Softwood

In softwood, the principal cell or fiber element is the tracheids. Tracheids, average between 3 to 5 mm in length, constitute over 90 % of the volume of most softwood.

The length to width ratio often exceeds 100:1. In India, softwood is found only at higher altitude (2,400 to 3,000 meters above sea level) (TAPPI, 2001).

**Hardwood**

The use of the hardwood is increasing in pulp industry. Some of the important ones are *Eucalyptus, Poplars, Beech,* etc. The wood fibers, which are predominantly cell type, are elongated, thick walled fibers having an average length slightly over 1mm and an average diameter of about 0.02mm (TAPPI, 2001).

**Percentage of cellulose, hemicellulose and lignin in softwood and hardwood** (TAPPI, 2001).

| Structural polysaccharides | Softwood | Hardwood |
|---|---|---|
| Cellulose | 42±2 | 45±2 |
| Hemicellulose | 27±2 | 30±5 |
| Lignin | 28±3 | 20±4 |

These parameters are very important for the paper and pulp industry.

## Cellulose

It is a high molecular weight, linear polymer of repeating beta –D glucopyranose units. It is the chief structural element and major constituent of the cell wall of trees and plants. The cellulose fiber is hollow and the wall of the fiber consists of several layers, which are build up of fibrils. As cellulose is a polyhydroxy compound, the many hydroxyl groups allow for the intermolecular hydrogen bonding which hold the fiber together in paper-no other adhesive is necessary. So high cellulose content is desired in the wood (TAPPI, 2001). *Eucalyptus tereticornis* (5-6 yr. Old) contains 42.4% of cellulose (Singh *et al*. 1987).

## Hemicellulose

Hemicellulose is not a single substance but a collective name for various low molecular weight polysaccharides associated with cellulose in the cell wall. Hemicellulose is polysaccharides, composed of relatively few different monomer units, the most common of which is D-xylose, D-mannose, D-galactose, L-arabinose, 4-O-methyl-D-glucronic acid, D-galacturonic acid, and D-glucucronic acid.

**Composition of hemicelluloses of *E. tereticornis*** (Singh *et.al*. 1971)

| Species | Xylose (%) | Arabinose (%) | Uronic acids (%) |
|---|---|---|---|
| *E. tereticornis* | 76.1 | 0.71 | 21.6 |

The molecules consist of a linear chain, with short (one or two units) side chains (Timell, 1967). They serve as a supportive matrix for the cellulose microfibrils and appear to have relatively uniform distribution. Hemicellulose is hydrophilic (water loving) and plays a major role in fiber's ability to absorb water during beating and refining. Consequently, they promote internal lubrication of the fiber, leading to improved flexibility, ease of mechanical lubrication, and increased sheet density (Haun, 1970).

Nordman *et al*. (1966) found that the intrinsic bonding strength of well beaten pulp sheets with high hemicellulose content was progressively much stronger, up to twice as much, when the pressure used for consolidating the wet sheet was reduced from 40 to 0.2 kg/cm$^2$. They also found that the intrinsic bonding strength was lower in sheets made from pulp with lower hemicellulose content.

## Lignin

Lignin is a large complex molecule of undetermined configuration and construction. It differs with different species of wood and plants. However, it is constructed mainly of phenylpropane units. These are benzene ring to an end of which is attached a string of three carbon atoms. Not all lignin are chemically equivalent. In softwood, for example, the lignin in the secondary cell wall can have twice the number of phenolic

groups as the middle lamella lignin (TAPPI, 2001). Lignin is hydrophobic and its presence, especially if above about 4% in wood pulp, tends to bind the interior elements or fibrils of the fibers together and detracts from inter fiber bonding during the process of papermaking. Lignin is normally removed from the pulp by bleaching. It results in a stronger paper by making the fiber more flexible and by permitting easier fibrillation. However if the extraction process is carried to extreme greater proportion of hemicelluloses are removed with the lignin and the cohesion of the paper is reduced (Clark, 1985). *Eucalyptus tereticornis* (5-6 yr. old) has 32.1% acid insoluble lignin (Singh *et al.,* 1987).

## Ash

Ash consists of the metallic ions sodium, potassium, calcium and the corresponding anions of carbonate, phosphate, silicate, sulfate, chloride, etc. remaining after the controlled combustion of wood. Wood ash is sufficiently alkaline so that when added to triglycerides it can be used to make soap (Biermann, 1993). No study has been undertaken to study the properties of wood or wood quality in tree species grown in degraded soils or soil contaminated or amended with industrial solid wastes.

# 3. Material and Methods

## Procurement and processing of wood samples

Wood samples of 6 year old *Eucalyptus tereticornis* were procured from village Durgapur, Distt. Dhenkanal, Orissa. They were grown in the soil amended with (ESP) fly ash @ 0%, 6%, 12%, 18%, and 24% v/v basis, involving 5 different treatments viz. $T_1$-$T_5$. $T_1$ is control containing only fly ash from 0-24%, $T_2$ is microbial consortium of mycorrhizal fungus (*Aspergillus*), free-living nitrogen fixing bacteria (*Azotobacte*r) and phosphate solubilizing microbes (*Pisolithus*), $T_3$ is soil conditioner (composted saw dust), $T_4$ is soil conditioner + microbial inoculants and $T_5$ involved addition of chemical fertilizers diammonium phosphate (DAP) @ 17.5g/plant and muriate of potash (MOP) @ 5g/plant at the time of plantation. Urea application @ 50 g/plant was given after three months of plantation. The soil on which plantation was established was partially degraded with soil belonging to order Ultisols having reddish brown lateritic characteristics. The soil texture was clayey. Samples from different treatments were debarked and pulpable wood was dried and crushed in ara machine and wood dust was collected which was further crushed in a mixer grinder and sieved using 0.2 mm sieve and used for analysis of secondary polysaccharides cellulose, hemicellulose and lignin and heavy metals Cu, Cr, Fe, Ni and Pb.

## Estimation of secondary polysaccharides in wood

### Estimation of cellulose

Cellulose was estimated as per the procedure of Updeggraff, 1969.

### Reagents

1. Sulfuric acid (67%) –67 ml of con. $H_2SO_4$ was diluted in 33 ml. of water.
2. Standard Solution–50 mg micro-crystalline cellulose was dissolved in 100 ml of 67% $H_2SO_4$ with gentle heat. The volume was made up to 500 ml with distilled water to give 100 µg/ml standard solution.

3. Acetic-nitric (A-N) acid reagent –150 ml of 80% acetic acid was mixed with 15 ml of con. $HNO_3$.
4. Anthrone reagent –0.2 gm anthrone was dissolved in 100 ml of chilled con. $H_2SO_4$ preserved in ice-bath.

**Procedure**

1. 0.2 gm of the sieved wood sample was accurately weighed and 10 ml of acetic-nitric reagent was added and then the tube was covered with marble.
2. The sample was digested in boiling water for one hour.
3. The digest was centrifuged (8-10 K rpm, 10 minutes). The supernatant was discarded and the pellet was washed 2-3 times, each time with 10 ml of distilled water.
4. 10 ml of 67% $H_2SO_4$ was added to the pellet, mixed well and the pellet was dissolved at room temperature and left for 1 hour. The solution was diluted (100-1000 fold) with distilled water.
5. 0-100 μg standard cellulose solution was taken in the test tubes and the volume was made to 1 ml with distilled water. The sample solution was diluted appropriately and 1.0 ml was taken.
6. All the tubes were placed in ice-bath for about 10-15 minutes and then 5.0 ml of chilled anthrone reagent was added (to be prepared fresh daily) to each tube.
7. All the tubes were placed in boiling water bath for 15 min and then cooled to room temperature and O.D. was read at 620 nm.
8. Standard curve was prepared and cellulose content in the sample was estimated.

**Estimation of Hemicellulose (Pentosans)**

Hemicellulose (pentosans) was estimated as per the procedure of Deschatelets, 1986.

**Reagents**

1. Standard 0.1% xylose solution in distilled water.
2. (3 % W/V) $H_2SO_4$
3. 10 (N) NaOH: 400 gm NaOH in 1 liter distilled water.

**Procedure**

1. To 2.0 gm of sieved wood sample, 20 ml of (3% W/V) $H_2SO_4$ was added and was autoclaved at 15 psi, $121^0$C for 1 hr.
2. To the sample 50 ml distilled water was added and the pH was adjusted to 7.0-7.5 with 10 N NaOH. Volume was made up to 100 ml with distilled water and filtered through Whatman No. 1 filter paper.
3. 0-500 µg standard xylose solution was taken in the test tubes and the volume was adjusted to 1.0 ml. The digested filtrate of the wood sample was diluted appropriately, and 1.0 ml was taken (avoided exposure to light in the following steps).
4. 5.0 ml of p-bromo-aniline was added to the filtrate and incubated in water bath at $70^0$C for 10 minutes. Subsequently incubated at room temperature for 70 minutes and O.D. was read at 540 nm.
5. The standard curve was prepared and the hemicellulose (pentosans) content was calculated in the wood sample.

**Estimation of acid insoluble lignin**

Estimation of acid insoluble lignin was done as per the procedure of TAPPI test methods, 1989.

**Reagents**

72% $H_2SO_4$ solution – 665 ml of concentrated $H_2SO_4$ was carefully poured into 300 ml of water, and after cooling, made up the volume to 1000 ml.

**Procedure**

1. To the beakers containing 1.0 gm of sieved wood sample, 15 ml of cold (10 to $15^0$C) 72% $H_2SO_4$ was added. The acid was added gradually in small increments while stirring and macerating the material with a glass rod. The beaker was kept in a bath at 2 $\pm 1^0$C during the dispersion of the material.
2. After the sample was dispersed, the beaker was covered with watch glass and was kept in a bath at 20 $\pm$ $1^0$C for 2 hours. The material was stirred frequently during this time to ensure complete dissolution.
3. 300 to 400 ml of water was added to a flask and the material was transferred from the beaker to the flask containing water and was rinsed and diluted with water to 3% concentration of sulfuric acid, to a total volume of 575 ml.
4. The solution was boiled for 4 hrs, maintaining constant volume by using a reflex condenser.
5. The insoluble material (lignin) was allowed to settle, by keeping the flask in an inclined position.
6. Without stirring up the precipitate, the supernatant solution was filtered through a filtering crucible. Then the lignin was transferred quantitatively to the filter, using hot water and a rod with rubber policeman.
7. The lignin was washed free of acid with hot water.
8. The crucible (having lignin) was dried in an oven at 105 $\pm$ $3^o$C to a constant weight. The crucible was cooled in a desiccators and weighed.

**Calculation**

Lignin % = A 100/W  where, A = weight of lignin
W = weight of sample

**Estimation of ash**

Ash content was estimated as per the procedure of Indian standard no. 10158: 1982.

**Procedure**

1. 1 gm of wood sample was taken in a silica crucible and was incinerated for 2 hours in a muffle furnace at 600°C.
2. After 2 hours, the crucible was taken out, cooled to ambient temperature in a desiccator and the final weight was taken.
3. Thereafter, residual inorganic ash content was determined

Ash (%) = [(initial wt. – final wt.) / sample wt.] x 100

## Estimation of heavy metal in wood sample

Estimation of heavy metal in wood sample as per the procedure of TAPPI Test Methods, 1989.

**Reagents**

1. Concentrated $HClO_4$ and $HNO_3$.
2. HCl (50%) – Dilute conc. HCl with water in 1:1 ratio.

**Procedure**

1. 1gm of sieved wood sample was taken in a 150 ml beaker.
2. 20 ml of $HNO_3$ and $HClO_4$ was added in 3:1 ratio.

3. The sample was digested on a hot plate at a temperature corresponding to $100^0$C for 3-4 hours until a whitish brown dry mass was obtained.
4. The residue was suspended in 50% HCl and volume was made up to 50 ml.
5. The solution was filtered through Whatman No. 42 filter paper.
6. The filtrate was analyzed for metal content using Atomic Absorption Spectrophotometer (*GBC 932AA*).

| Metal | Sensitivity μg/ml |
|---|---|
| Cu | 0.025 |
| Cr | 0.05 |
| Fe | 0.05 |
| Ni | 0.04 |
| Pb | 0.06 |

# Statistical analysis

## Analysis of Variance (ANOVA)

Analysis of variance (ANOVA) is a useful technique for comparison of several groups. R.A. Fisher (Rao, K. V., 1996) originally developed the method of analysis of variance in the 1920s. Two- way analysis of variance is utilized when there is need to study the effect of two factors on variations in a specific variable. The assumptions made in this type of ANOVA are that (i) the subjects must be chosen at random (ii) the variable under study must have normal characteristics, (i.e., coefficient of skewness is equal to zero and coefficient of kurtosis is equal to three), (iii) variances between comparable groups are mostly same or homogenous. Two-way ANOVA is utilized for the experimental designs like the randomized complete block design.

| SL No. | Source | Sum of squares | Degrees of freedom | Mean sum of squares (MSS) | Variance ratio "F" |
|---|---|---|---|---|---|
| I | Blocks | $SS_{Blocks}$ | (n-1) | $\frac{SS_{Blocks}}{n-1}$ | $F_1 = \frac{MS_{blocks}}{MS_{Error}}$ |
| Ii | Treatments | $SS_{Treatments}$ | (k-1) | $\frac{SS_{Treatments}}{(k-1)}$ | $F_2 = \frac{MS_{Treatments}}{MS_{Error}}$ |
| Iii | Interaction | $SS_{Interaction}$ | (n-1) (k-1) | $\frac{SS_{Interaction}}{(n-1)(k-1)}$ | $F_3 = \frac{MS_{Intearction}}{MS_{Error}}$ |
| Iv | Error | $SS_{Error}$ | Nk (m-1) | $\frac{SS_{Error}}{nk(m-1)}$ | |
| V | Total | $SS_{Total}$ | nkm-1 | | |

N- Total number of observations

n- Number of blocks

k-Number of treatments

m-Number of replicates

$F_1$-Variance ratio for blocks with df of (n-1) vs. nk (m-1)

$F_2$- Variance ratio for treatments with df of (k-1) vs nk (m-1)

$F_3$-Variance ratio for interaction with df of (n-1) (k-1) vs nk (m-1)

These values can be compared with F values for their degrees of freedom at 5 percent or at 1 percent level of significance. If the calculated values are higher than their critical values at 5 percent or 1 percent level, it is an indication of significance. If the calculated values are lower than their critical values for their degrees of freedom, it is an indication that there are no significant differences.

i.e., If $F_1 > F_{0.05}$ then the probability of significance is $P < 0.05$

If $F_1 > F_{0.01}$ then the probability of significance is $P < 0.01$

If $F_1 < F_{0.05}$ then the probability of significance is $P > 0.05$ (not significant)

If $F_2 > F_{0.05}$ then the probability of significance is $P < 0.05$

If $F_2 < F_{0.05}$ then the probability of significance is $P > 0.05$ (not significant)

## The Least Significant Difference (LSD)

ANOVA test rarely provides an adequate basis for drawing inferences. If the ANOVA test shows significance, further analysis of data is necessary to determine which of the treatments differ. The least significant difference procedure is simply the well known student's test.

$$LSD = t_{\alpha} \sqrt{2S^2/n}$$

Where α is the level of significance. "α" is usually chosen as 5 percent, 1 percent or 0.1 percent level. $t_{\alpha}$ value is taken from the table of t, with α level of significance and degrees of freedom of the error.

$S^2$ is the mean error variance and

n is the sample size of each group.

LSD value is calculated at 5 percent, 1 percent or 0.1 percent level. If difference in the mean values of two groups is greater than the LSD value at a given level of significance, then they are significantly different from each other at that level of significance.

# 4. Results and Discussion

## Secondary polysaccharides

### Cellulose

Cellulose content in all the 75 debarked wood samples of *Eucalyptus tereticornis* samples was estimated. It varied from 26.6 to 57.0% (Table 1a). In treatment $T_1$ it varied from 33.6-45.3%, in $T_2$ from 32.6-46.9%, in treatment $T_3$ it ranged from 35.3-42.8%, in $T_4$ it varied from 37.7-47.7%, whereas in treatment $T_5$ it ranged from 34.5-47.0%. Overall cellulose content was observed to be minimum at 24% fly ash (35.8%) and maximum at 12% fly ash, i.e. 45.4% (Table 1b). At 0% fly ash, minimum cellulose content was observed in $T_4$ treatment and maximum in $T_1$, at 6% fly ash it was observed to be minimum in $T_3$ and maximum in $T_1$ treatment, at 12% fly ash it was found to be minimum in $T_3$ treatment and maximum in $T_4$ treatment, at 18% fly ash cellulose content was observed to be minimum at $T_5$ treatment and maximum at $T_1$ treatment, whereas at 24% fly ash it was found to be minimum at $T_2$ and maximum at $T_4$ treatment. Overall among all the treatments cellulose content was observed to be minimum in $T_3$ (39.0%) and maximum in $T_1$ treatment, i.e. 41.8% (Table 1b). *Eucalyptus tereticornis* has been reported to contain 42.4% cellulose (Singh *et al*., 1987).

Statistical analysis (ANOVA) showed that different fly ash concentrations had significant effect on the cellulose content in the debarked wood samples, but the different treatments, i.e. $T_1$-$T_5$ had no such significant effect on the cellulose content (Table 10). LSD showed that the maximum cellulose content was observed at 12% fly ash but this was at par with control. The effect of fly ash amendment up to 18% on cellulose content was not significant Cellulose is the major structural element of cell wall of tress and plants. Therefore, soil amendment with fly ash up to 18% did not have any detrimental effect on the cellulose content of *Eucalyptus tereticornis,* but at 24% fly ash cellulose content decreased significantly (Table 11a).

**Hemicellulose (pentosans)**

Hemicellulose content of the debarked and pulpable wood samples varied from 7.1-13.0% (Table 2a). In treatment $T_1$ hemicellulose content varied from 9.0-10.2%, in $T_2$ it varied from 9.1-9.8%, in $T_3$ it ranged from 9.2-10.0%, in treatment $T_4$ hemicellulose content ranged from 8.8-9.6%, whereas in $T_5$ treatment it ranged from 8.5-10.1%. Overall hemicellulose content was observed to be minimum at 0% fly ash (9.1%) and maximum at 12% concentration of fly ash, i.e. 9.9% (Table 2b). At 0% fly ash minimum hemicellulose content ranged from 8.5-9.6%, at 6% fly ash it ranged from 9.1-10.0 %, at 12% fly ash hemicellulose content ranged from 9.1-10.0%, at 18% fly ash hemicellulose content varied from 8.8-10.2% whereas at 24% fly ash it ranged from 9.4-10.0%. Among different treatments minimum hemicellulose content was observed in $T_4$ treatment (9.3%) and maximum in $T_1$ treatment, i.e. 9.7% (Table 2b).

As per the results of statistical analysis (ANOVA) mean differences between the different fly ash concentrations and different treatments were not significant. Hemicellulose mainly serves as a supportive matrix for the cellulose microfibrils and appears to have relatively uniform distribution. Results showed that there was no negative impact of fly ash when added at 0-24% v/v in soil at the time of plantation on hemicellulose content and in various treatments it was at par with control (Table 10).

**Lignin**

Lignin content was in the range from 26.6-49.8% (Table 3a). In treatment $T_1$ and $T_2$ lignin was observed to be minimum at 0% fly ash and maximum at 18%, in $T_3$ it was minimum at 0% and maximum at 6%. In treatment $T_4$ lignin content was found to be minimum at 0% and maximum at 18%, whereas in $T_5$ treatment it was found to be minimum at 24% and maximum at 18%. Overall among different fly ash concentrations lignin content was observed to be minimum at 0% fly ash (31.3%) and maximum at 18% fly ash, i.e. 37.8% (Table 3b). At 0% fly ash, lignin varied from 29.7-32.9%, at 6% it ranged from 31.9-37.5% and at 12% it varied from 32.0-34.4%.

At 18% fly ash lignin content varied from 35.2-40.6%, whereas at 24% fly ash it varied from 31.0-35.6%. Overall among all the treatments lignin content was observed to be minimum in $T_5$ treatment (32.9%) and maximum in $T_4$ treatment, i.e. 34.5% (Table 3b). Five to six years old *Eucalyptus tereticornis* had 32% acid insoluble lignin (Singh *et al*., 1987).

Statistical analysis showed (ANOVA) that mean response between different fly ash concentrations was significant whereas there was no effect of any treatment on the lignin content (Table 10). LSD test showed that significant increase in lignin content was observed at 6% and 18% of fly ash. Soil amendment with fly ash at 12% and 24%, did not show any significant increase or decrease in the lignin content when compared with control (Table 11a).

**Ash**

Ash content in all the debarked and pulpable wood samples varied from 2.2-5.6% (Table 4a). In $T_1$ treatment ash content varied from 2.9-3.9%, in $T_2$ it ranged from 3.1-4.3%, in $T_3$ ash content varied from 3.4-3.8%. In $T_4$ treatment ash content varied from 3.1-3.9%, whereas in $T_5$ treatment it ranged from 3.1-4.5%. Overall among different fly ash concentrations ash content varied from 3.5-3.8% (Table 4b). At 0% fly ash, ash content varied from 3.1-4.2%, at 6% it ranged from 2.9-4.5%, at 12% ash content varied from 3.2-4.2%. At 18% concentration of fly ash, ash content varied from 3.1-3.8%, whereas at 24% fly ash it ranged from 3.2-4.3%. Among different treatments ash content varied from 3.5-3.8% (Table 4b).

Statistical analysis (ANOVA) showed that neither fly ash nor the treatment had any effect on the ash content of debarked wood of *Eucalyptus tereticornis* (Table 10).

## Heavy Metals

### Cu

Cu concentration in all the 75 debarked and pulpable wood samples of *Eucalyptus tereticornis* varied from 1.1-5.9 mg/Kg (Table 5a). In treatments, $T_1$, $T_2$, $T_3$ and $T_5$ Cu concentration was observed to be minimum at 6% fly ash and maximum at 18% fly ash, whereas in $T_4$ it was minimum at 12% and maximum at 18% fly ash. Among different concentrations of fly ash Cu concentration was found to be minimum at 6% fly ash (2.0 mg/Kg) and maximum at 18% fly ash, i.e. 3.2 mg/Kg (Table 5b). At 0% fly ash Cu concentration ranged from 1.8-2.5 mg/Kg, at 6% fly ash it was from 1.6-2.6 mg/Kg, at 12% fly ash it ranged from 1.7-2.4, at 18% fly ash Cu concentration was from 2.9-3.8 mg/Kg and at 24% fly ash it ranged from 2.4-2.9 mg/Kg. Overall, Cu concentration was found to be minimum in treatment $T_5$ (2.2 mg/Kg) and maximum in $T_2$ and $T_4$, i.e. 2.6 mg/Kg (Table 5b).

Statistical analysis (ANOVA) showed that different fly ash concentrations had significant effect on the concentration of Cu in the debarked wood samples, but the different treatments, i.e. $T_1$-$T_5$ had no such significant effect on the Cu concentration (Table 10). LSD test showed that among different fly ash concentrations, there was no significant increase in Cu concentration at 6%, 12% and 24% fly ash as compared to control, maximum concentration of Cu was observed at 18% fly ash (Table 11b).

### Cr

Cr concentration in all the 75 samples varied from 4.1 to 19.0 mg/Kg (Table 6a). In $T_1$ treatment Cr concentration was observed to be minimum at 0% fly ash and maximum at 18% and 24% fly ash. In the treatments $T_2$ and $T_3$, Cr concentration was observed to be minimum at 0% and maximum at 24% fly ash. In $T_4$ minimum Cr concentration was observed at 0% and maximum at 18% fly ash whereas in $T_5$ it was minimum at 6% and maximum at 18% fly ash. Overall among different fly ash concentration Cr concentration was observed to be minimum at 0% fly ash (5.9 mg/Kg) and maximum at 18%, i.e. 16.8 mg/Kg (Table 6b). At 0% concentration

of fly ash, Cr concentration ranged from 5.6 to 15.6 mg/Kg, at 6% fly ash Cr concentration ranged from 7.7-9.2 mg/Kg, at 12% fly ash it ranged from 12.0-15.0 mg/Kg, at 18% fly ash Cr concentration was from 15.9-17.6 mg/Kg and at 24% fly ash it ranged from 15.3-17.1 mg/Kg. Overall Cr concentration was found to be minimum in $T_1$ treatment and maximum in $T_3$ treatment, i.e. 12.6 mg/Kg (Table 6b). Cr concentration in the stem of *Cannabis sativa* was reported to be <0.1 mg/Kg when grown in Cr contaminated soil (Citterio *et al.,* 2003).

Statistical analysis (ANOVA) showed that different fly ash concentrations had significant effect on the concentration of Cr in the debarked wood samples, but the different treatments, i.e. $T_1$-$T_5$ had no such significant effect on the Cr concentration (Table 10). LSD test showed that there was significant increase in Cr concentration corresponding with increase in fly ash concentration with maximum Cr concentration at 18% and 24% of fly ash (Table 11b).

**Fe**

Fe concentration varied from 79.5 to 322.0 mg/Kg in all the 75 wood samples (Table 7a). In $T_1$ treatment minimum Fe concentration was observed at 24% fly ash and maximum at 18% fly ash. In $T_2$ minimum Fe concentration was observed at 6% fly ash and maximum at 12% and 18% fly ash. In $T_3$ treatment Fe was minimum at 24% and maximum at 6% fly ash. In $T_4$ Fe was found to be minimum at 24% and maximum at 18%, whereas in $T_5$ treatment it was observed to be minimum at 24% and maximum at 18% fly ash. Overall, Fe concentration was observed to be minimum at 24% fly ash (138.6 mg/Kg) and maximum at 18%, i.e. 225.9 mg/Kg (Table 7b). At 0% fly ash, Fe concentration was observed to be minimum in $T_3$ and maximum in $T_1$ treatment. At 6% fly ash Fe concentration was observed to be minimum in $T_2$ and maximum in $T_3$ treatment. At 12% fly ash Fe concentration was found to be minimum in $T_5$ and maximum in $T_2$ treatment. At 18% fly ash minimum Fe was observed in $T_3$ and maximum in $T_4$ treatment, whereas at 24% fly ash it was found to be minimum in $T_5$ and maximum in $T_1$ treatment. Overall among different treatments

Fe concentration was observed to be minimum in $T_5$ (175.8 mg/Kg) and maximum in $T_1$, i.e. 198.0 mg/Kg (Table 7b).

Statistical analysis (ANOVA) showed that different fly ash concentrations had significant effect on the concentration of Fe in the debarked wood samples, but the different treatments, i.e. $T_1$-$T_5$ had no such significant effect on the Fe concentration (Table 10). LSD test showed that there was no significant increase in the Fe concentration up to 12% of fly ash, when compared with control, while lowest concentration of Fe was observed at 24% of fly ash and highest Fe concentration was observed at 18% fly ash (Table 11b).

**Ni**

Ni concentration in all the 75 samples varied from 0.2-16.6 mg/Kg (Table 8a). In $T_1$, $T_2$, $T_3$, $T_4$ and $T_5$ treatments minimum Ni concentration was observed at 0% fly ash and maximum at 24% fly ash. Overall among different fly ash concentrations minimum Ni concentration was observed at 0% fly ash (1.8 mg/Kg) and maximum at 24% fly ash, i.e. 14.5 mg/Kg (Table 8b). At 0% concentration of fly ash Ni concentration varied from 1.3-2.6 mg/Kg, at 6% fly ash it ranged from 3.2-3.8 mg/Kg, at 12% fly ash from 3.9-5.8 mg/Kg, at 18% fly ash Ni concentration varied from 6.7-8.6 mg/Kg whereas at 24% it ranged from 14.0-15.3 mg/Kg. Overall among all the treatments it was found to be minimum in $T_1$ treatment (6.0 mg/Kg) and maximum in $T_4$ treatment, i.e. 7.2 mg/Kg (Table 8b). Ni concentration in the stem of *Cannabis sativa* has been reported to range from 0.1-14.0 mg/Kg when grown in Ni contaminated soil (Citterio *et al*., 2003).

ANOVA test showed that although there was some increase in Ni concentration in different treatments when compared with control, with maximum Ni concentration observed in $T_4$ treatment (microbes + SC) but this increases was not statistically significant whereas difference among the different fly ash concentrations was found to be significant (Table 10). LSD test showed that there was significant increase in Ni

concentration with corresponding increase in fly ash concentration. Maximum Ni concentration was observed at 24% of fly ash (Table 11b).

**Pb**

Pb concentration in all the 75 wood samples varied from 1.9 to 23.6 mg/Kg (Table 9a). In treatment $T_1$, $T_2$, $T_3$ and $T_4$ minimum Pb concentration was observed at 0% fly ash and maximum at 18% fly ash, whereas in $T_5$ treatment it was found to be minimum at 0% and maximum at 12% fly ash. Overall, Pb concentration was observed to be minimum at 0% (6.2 mg/Kg) fly ash and maximum at 18%, i.e. 19.3 mg/Kg (Table 9b). At 0% fly ash, Pb concentration varied from 3.7-8.8 mg/Kg, at 6% it ranged from 8.3-12.0 mg/Kg, at 12% fly ash it varied from 12.3-18.0 mg/Kg, at 18% fly ash Pb concentration varied from 17.3-21.1 mg/Kg, where as at 24% fly ash it ranged from 14.0-16.6 mg/Kg. Overall among all the treatments it was observed to be minimum in $T_3$ treatment (12.4 mg/Kg) and maximum in $T_5$ treatment, i.e. 13.8 mg/Kg (Table 9b). Pb concentration in the stem of white poplar trees from spill-affected sites in the riparian forest of the Guadiamar River was reported to be in the range of 1.54-2.06 mg/Kg (Madejon *et al*., 2004). In general, Pb is rather immobile in soil and easily forms organic complexes with fulvic acids.

ANOVA test showed that there was no effect of any treatment on Pb concentration in wood whereas different fly ash concentrations had significant effect on Pb concentration in wood (Table 10). LSD test showed that Pb concentration increased significantly with corresponding increase in fly ash concentration up to 18% of fly ash. Maximum Pb concentration was observed at 18% fly ash (Table 11b).

It has been reported that *Quercus ilex* L. phytomas from stem, leaf and root acts as adsorbent of Cr, Ni, Cu, Cd and Pb. The metal uptake capacity of the stem for different metals was found to be in the order of Ni> Pb> Cu> Cd> Cr (Prasad and Freitas, 2000). A number of plant species grown as biomass fuel crops have been found to take up heavy metals, frequently in high concentration. The biomass fuel crop species identified are *Salix, Miscanthus, Phalaris* and *Eucalyptus* (Sanghi and

Sasi, 2001). There are several advantages to the use of biomass fuel crops over other approaches to bioremediation. High productivity and the production of large quantities of biomass and also high economic return from the agricultural land. The rapid year-round growth of *Eucalyptus* is advantageous for the elemental uptake and immobilization needed in phytoremediation. *Eucalyptus* production combined with waste water recycling has many mutual advantages, such as increasing tree growth, recycling nutrients and renovating wastewater while at the same time producing mulch, pulpwood and energywood (Warrag *et al.,* 1988).

# 5. Conclusions

1. Among various structural polysaccharides, cellulose in pulpable wood was in the range of 32.6-47.7%, hemicellulose was in the range of 8.5-10.2%, lignin was in the range of 29.7- 40.6% and ash was in the range of 2.9-4.5%. Fly ash at 12% (v/v) addition in soil had no detrimental effect on any of these polysaccharide components of wood, rather caused an increase in cellulose content, whereas lignin content remained almost normal which was at par with wood grown in soils without fly ash. The ash and hemicellulose content in wood did not vary significantly.
2. Heavy metals (Cu, Cr, Fe, Ni, and Pb) varied significantly in *Eucalyptus* wood grown in soil with different fly ash concentrations. Overall Cu ranged from 1.6-3.8 mg/Kg, Cr ranged from 5.6-17.6 mg/Kg, Fe ranged from 112.8 to 248.3 mg/Kg, Ni ranged from 1.3-15.3 mg/Kg and Pb ranged from 3.7-21.1 mg/Kg. With increased addition of fly ash at 18-24% level the uptake of metal by wood was increased, however at 12% fly ash these metals were more or less in the range at par with wood grown in soil without fly ash. The order of metal uptake by wood in almost in all treatments was Fe>Pb>Cr>Ni>Cu.
3. No correlation could be established between structural polysaccharides and heavy metal content of wood. Obviously with increased biomass production or growth there is bound to have higher synthesis of polysaccharides with concomitant higher uptake of heavy metals from soil provided they are available in soil solution at higher level. Certainly at higher doses of fly ash i.e. 18-24% the metal content is also increased in soil. Fly ash addition in soil has been reported to cause increase in electrical conductivity of soil which is directly due to increased availability of soluble salts (Goyal *et.al*., 2002, Jala and Goyal, 2004 a, b).
4. 12% fly ash from ESP appears to be an optimum dose, which can be judiciously added to soil in order to improve yield and cellulose content of wood.

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**Table 1a: Cellulose content (%) in *Eucalyptus* wood grown in soil amended with fly ash and under different treatments**

| Fly ash (%) | $T_1$ (control) | | | $T_2$ (microbes) | | | $T_3$ (soil conditioner) | | | $T_4$ (microbes+SC) | | | $T_5$ (fertilizer) | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | $R_1$ | $R_2$ | $R_3$ | $R_1$ | $R_2$ | $R_3$ | $R_1$ | $R_2$ | $R_3$ | $R_1$ | $R_2$ | $R_3$ | $R_1$ | $R_2$ | $R_3$ |
| $F_0$ (control) | 41.9 | 42.6 | 44.5 | 40.2 | 42.6 | 43.6 | 46.1 | 42.3 | 39.8 | 40.2 | 37.0 | 42.7 | 42.2 | 34.4 | 45.6 |
| $F_1$ (6%) | 42.4 | 42.0 | 51.5 | 42.3 | 40.9 | 43.8 | 34.7 | 26.6 | 44.5 | 34.1 | 37.8 | 45.6 | 35.3 | 47.3 | 43.9 |
| $F_2$ (12%) | 53.0 | 48.6 | 33.2 | 49.4 | 44.4 | 46.8 | 43.2 | 36.0 | 42.2 | 45.1 | 41.0 | 57.0 | 46.0 | 41.1 | 53.8 |
| $F_3$ (18%) | 45.9 | 33.1 | 47.0 | 37.2 | 34.8 | 44.0 | 36.4 | 39.4 | 43.5 | 40.0 | 31.2 | 42.0 | 31.4 | 34.7 | 37.6 |
| $F_4$ (24%) | 29.7 | 32.4 | 38.8 | 26.8 | 33.4 | 37.7 | 36.3 | 30.5 | 43.0 | 28.4 | 51.4 | 41.6 | 36.1 | 30.9 | 41.0 |

**Table 1b: Mean cellulose (%) ± SE**

| Fly ash (%) | $T_1$ (control) | $T_2$ (microbes) | $T_3$ (soil conditioner) | $T_4$ (microbes+SC) | $T_5$ (fertilizer) | M±S.E. |
|---|---|---|---|---|---|---|
| $F_0$ (control) | 43.0±0.8 | 42.1±1.0 | 42.8±1.8 | 40.3±1.4 | 40.7±3.3 | 41.8±0.5 |
| $F_1$ (6%) | 45.3±3.1 | 42.3±0.8 | 35.3±5.2 | 39.2±3.4 | 42.2±3.6 | 40.9±1.7 |
| $F_2$ (12%) | 44.9±6.0 | 46.9±1.4 | 40.4±2.2 | 47.7±4.8 | 47.0±3.7 | 45.4±1.3 |
| $F_3$ (18%) | 42.0±4.5 | 38.7±2.8 | 39.8±2.1 | 37.7±3.3 | 34.5±1.8 | 38.5±1.2 |
| $F_4$ (24%) | 33.6±2.7 | 32.6±3.2 | 36.6±3.6 | 40.4±6.7 | 36.0±2.9 | 35.8±1.4 |
| M±S.E. | 41.8±0.5 | 40.5±2.4 | 39.0±1.4 | 41.0±1.7 | 40.1±2.2 | |

**Table 2a: Hemicellulose content (%) in E*ucalyptus* wood grown in soil amended with fly ash and under different treatments**

| Fly ash (%) | $T_1$ (control) | | | $T_2$ (microbes) | | | $T_3$ (soil conditioner) | | | $T_4$ (microbes+SC) | | | $T_5$ (fertilizer) | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | $R_1$ | $R_2$ | $R_3$ | $R_1$ | $R_2$ | $R_3$ | $R_1$ | $R_2$ | $R_3$ | $R_1$ | $R_2$ | $R_3$ | $R_1$ | $R_2$ | $R_3$ |
| $F_0$ (control) | 10.7 | 7.7 | 8.7 | 10.5 | 7.9 | 9.0 | 10.2 | 8.1 | 9.2 | 11.2 | 8.0 | 9.7 | 9.8 | 7.1 | 8.5 |
| $F_1$ (6%) | 7.5 | 10.0 | 10.8 | 7.2 | 9.9 | 10.2 | 8.2 | 10.5 | 11.2 | 7.2 | 10.6 | 11.0 | 9.5 | 10.2 | 9.0 |
| $F_2$ (12%) | 10.9 | 10.0 | 9.2 | 11.2 | 9.9 | 8.5 | 10.4 | 10.5 | 8.2 | 8.8 | 10.6 | 8.1 | 10.5 | 10.2 | 9.1 |
| $F_3$ (18%) | 8.6 | 13.0 | 9.0 | 8.6 | 11.8 | 8.4 | 8.5 | 11.3 | 8.3 | 8.1 | 10.4 | 7.8 | 8.7 | 12.5 | 9.1 |
| $F_4$ (24%) | 10.0 | 9.8 | 9.3 | 11.2 | 8.4 | 9.7 | 9.8 | 9.5 | 9.6 | 9.6 | 9.4 | 9.2 | 11.0 | 9.3 | 9.8 |

**Table 2b: Mean hemicellulose (%) ± SE**

| Fly ash (%) | $T_1$ (control) | $T_2$ (microbes) | $T_3$ (soil conditioner) | $T_4$ (microbes+SC) | $T_5$ (fertilizer) | M±S.E. |
|---|---|---|---|---|---|---|
| $F_0$ (control) | 9.0±0.9 | 9.1±0.8 | 9.2±0.6 | 9.6±0.9 | 8.5±0.8 | 9.1±0.2 |
| $F_1$ (6%) | 9.4±1.0 | 9.1±1.0 | 10.0±0.9 | 9.6±1.2 | 9.6±0.3 | 9.5±0.1 |
| $F_2$ (12%) | 10.0±0.5 | 9.8±0.8 | 9.7±0.7 | 9.1±0.8 | 9.9±0.4 | 9.9±0.2 |
| $F_3$ (18%) | 10.2±1.4 | 9.6±1.1 | 9.4±1.0 | 8.8±0.8 | 10.1±1.2 | 9.6±0.3 |
| $F_4$ (24%) | 9.7±0.1 | 9.7±0.8 | 9.6±1.0 | 9.4±0.1 | 10.0±0.5 | 9.7±0.1 |
| M±S.E. | 9.7±0.2 | 9.5±0.2 | 9.6±0.1 | 9.3±0.2 | 9.6±0.3 | |

**Table 3a: Lignin content (%) in *Eucalyptus* wood grown in soil amended with fly ash and under different treatments.**

| Fly ash (%) | $T_1$ (control) | | | $T_2$ (microbes) | | | $T_3$ (soil conditioner) | | | $T_4$ (microbes+SC) | | | $T_5$ (fertilizer) | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | $R_1$ | $R_2$ | $R_3$ | $R_1$ | $R_2$ | $R_3$ | $R_1$ | $R_2$ | $R_3$ | $R_1$ | $R_2$ | $R_3$ | $R_1$ | $R_2$ | $R_3$ |
| $F_0$ (control) | 32.3 | 28.8 | 34.3 | 30.9 | 26.6 | 31.5 | 31.8 | 28.6 | 34.9 | 30.6 | 29.0 | 32.0 | 32.8 | 30.9 | 34.9 |
| $F_1$ (6%) | 34.0 | 31.3 | 30.5 | 31.8 | 48.6 | 30.2 | 31.4 | 48.3 | 37.2 | 31.4 | 49.8 | 31.2 | 32.9 | 32.0 | 33.4 |
| $F_2$ (12%) | 31.4 | 33.1 | 32.8 | 31.7 | 34.7 | 32.1 | 32.8 | 31.2 | 32.0 | 33.0 | 36.2 | 34.1 | 32.0 | 34.8 | 30.9 |
| $F_3$ (18%) | 43.0 | 36.5 | 31.3 | 39.8 | 38.0 | 34.2 | 35.3 | 39.2 | 35.2 | 42.5 | 40.7 | 33.8 | 42.9 | 40.1 | 38.7 |
| $F_4$ (24%) | 34.0 | 35.3 | 31.3 | 36.2 | 35.5 | 35.0 | 35.5 | 29.3 | 31.5 | 34.8 | 30.6 | 27.7 | 35.3 | 30.2 | 31.8 |

**Table 3b: Mean lignin (%) ± SE**

| Fly ash (%) | $T_1$ (control) | $T_2$ (microbes) | $T_3$ (soil conditioner) | $T_4$ (microbes+SC) | $T_5$ (fertilizer) | M±S.E. |
|---|---|---|---|---|---|---|
| $F_0$ (control) | 31.8±1.6 | 29.7±1.5 | 31.8±1.8 | 30.5±0.9 | 32.9±1.2 | 31.3±0.6 |
| $F_1$ (6%) | 31.9±1.0 | 36.9±5.9 | 37.2±5.6 | 37.5±6.2 | 32.8±0.4 | 35.3±1.2 |
| $F_2$ (12%) | 32.4±0.5 | 32.8±0.9 | 32.0±0.5 | 34.4±0.9 | 32.6±1.2 | 32.9±0.4 |
| $F_3$ (18%) | 36.9±3.4 | 37.3±1.6 | 35.2±2.4 | 39.0±2.7 | 40.6±1.2 | 37.8±0.9 |
| $F_4$ (24%) | 33.5±1.2 | 35.6±0.4 | 31.5±2.0 | 31.0±2.1 | 32.4±1.5 | 32.8±0.8 |
| M±S.E. | 33.3±1.0 | 34.4±1.4 | 33.5±1.1 | 34.5±1.6 | 32.9±1.6 | |

**Table 4a: Ash content (%) in *Eucalyptus* wood grown in soil amended with fly ash and under different treatments.**

| Fly ash (%) | $T_1$ (control) | | | $T_2$ (microbes) | | | $T_3$ (soil conditioner) | | | $T_4$ (microbes+SC) | | | $T_5$ (fertilizer) | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | $R_1$ | $R_2$ | $R_3$ | $R_1$ | $R_2$ | $R_3$ | $R_1$ | $R_2$ | $R_3$ | $R_1$ | $R_2$ | $R_3$ | $R_1$ | $R_2$ | $R_3$ |
| $F_0$ (control) | 3.3 | 4.6 | 3.8 | 4.6 | 4.7 | 2.2 | 3.6 | 3.2 | 3.7 | 3.3 | 3.2 | 3.1 | 3.4 | 3.2 | 2.7 |
| $F_1$ (6%) | 3.5 | 2.2 | 3.1 | 3.9 | 4.2 | 4.1 | 3.2 | 3.8 | 3.5 | 3.2 | 4.3 | 3.9 | 5.3 | 5.2 | 3.1 |
| $F_2$ (12%) | 3.2 | 3.5 | 3.2 | 2.8 | 3.7 | 3.0 | 3.7 | 3.2 | 3.3 | 4.3 | 4.0 | 3.5 | 3.8 | 5.6 | 3.0 |
| $F_3$ (18%) | 3.7 | 4.3 | 3.4 | 2.8 | 3.0 | 3.6 | 4.0 | 4.4 | 2.4 | 2.6 | 4.2 | 2.7 | 3.5 | 3.1 | 4.5 |
| $F_4$ (24%) | 4.3 | 3.2 | 2.6 | 5.0 | 4.5 | 3.3 | 3.4 | 4.4 | 3.6 | 2.9 | 4.2 | 3.9 | 2.9 | 3.8 | 3.0 |

**Table 4b: Mean ash (%) ±SE**

| Fly ash (%) | $T_1$ (control) | $T_2$ (microbes) | $T_3$ (soil conditioner) | $T_4$ (microbes+SC) | $T_5$ (fertilizer) | M±S.E. |
|---|---|---|---|---|---|---|
| $F_0$ (control) | 3.9±0.4 | 4.2±0.8 | 3.5±0.1 | 3.2±0.07 | 3.1±0.2 | 3.6±0.2 |
| $F_1$ (6%) | 2.9±0.05 | 4.1±0.07 | 3.5±0.2 | 3.8±0.3 | 4.5±0.7 | 3.8±0.2 |
| $F_2$ (12%) | 3.3±0.07 | 3.2±0.3 | 3.4±0.2 | 3.9±0.2 | 4.2±0.8 | 3.6±0.2 |
| $F_3$ (18%) | 3.8±0.3 | 3.1±0.2 | 3.6±0.6 | 3.1±0.5 | 3.7±0.4 | 3.5±0.2 |
| $F_4$ (24%) | 3.4±0.5 | 4.3±0.5 | 3.8±0.3 | 3.7±0.4 | 3.2±0.3 | 3.7±0.2 |
| M±S.E. | 3.5±0.2 | 3.8±0.2 | 3.6±0.2 | 3.5±0.2 | 3.7±0.2 | |

**Table 5a: Cu concentration (mg/kg) in *Eucalyptus* wood grown in soil amended with fly ash and under different treatments**

| Fly ash (%) | $T_1$ (control) | | | $T_2$ (microbes) | | | $T_3$ (soil conditioner) | | | $T_4$ (microbes+SC) | | | $T_5$ (fertilizer) | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | $R_1$ | $R_2$ | $R_3$ | $R_1$ | $R_2$ | $R_3$ | $R_1$ | $R_2$ | $R_3$ | $R_1$ | $R_2$ | $R_3$ | $R_1$ | $R_2$ | $R_3$ |
| $F_0$ (control) | 3.4 | 2.3 | 1.9 | 3.7 | 2.0 | 1.4 | 2.0 | 2.8 | 1.6 | 2.2 | 2.8 | 1.6 | 1.9 | 1.8 | 1.6 |
| $F_1$ (6%) | 1.6 | 1.8 | 1.5 | 2.0 | 2.3 | 2.5 | 3.0 | 1.6 | 1.8 | 1.3 | 4.0 | 2.6 | 1.4 | 2.0 | 1.8 |
| $F_2$ (12%) | 1.8 | 1.4 | 2.6 | 1.9 | 1.2 | 4.0 | 1.6 | 1.5 | 4.2 | 1.1 | 1.6 | 2.4 | 1.4 | 1.8 | 2.4 |
| $F_3$ (18%) | 2.2 | 3.4 | 3.0 | 2.6 | 2.4 | 4.0 | 2.8 | 3.7 | 3.4 | 2.9 | 2.7 | 5.9 | 2.8 | 2.9 | 3.3 |
| $F_4$ (24%) | 3.0 | 2.6 | 2.9 | 3.4 | 2.4 | 2.8 | 2.5 | 2.8 | 2.0 | 2.4 | 2.5 | 2.7 | 2.6 | 2.8 | 2.6 |

**Table 5b: Mean Cu concentration ± SE**

| Fly ash (%) | $T_1$ (control) | $T_2$ (microbes) | $T_3$ (soil conditioner) | $T_4$ (microbes+SC) | $T_5$ (fertilizer) | M±S.E. |
|---|---|---|---|---|---|---|
| $F_0$ (control) | 2.5±0.4 | 2.4±0.7 | 2.1±0.4 | 2.2±0.3 | 1.8±0.1 | 2.2±0.1 |
| $F_1$ (6%) | 1.6±1.0 | 2.2±0.2 | 2.1±0.4 | 2.6±0.8 | 1.8±0.2 | 2.0±0.2 |
| $F_2$ (12%) | 1.9±0.4 | 2.4±0.9 | 2.4±0.9 | 1.7±0.4 | 1.9±0.3 | 2.0±0.1 |
| $F_3$ (18%) | 2.9±0.4 | 3.0±0.5 | 3.3±0.2 | 3.8±1.0 | 3.0±0.2 | 3.2±0.2 |
| $F_4$ (24%) | 2.8±0.1 | 2.9±0.3 | 2.4±0.2 | 2.5±0.1 | 2.7±0.1 | 2.7±0.1 |
| M±S.E. | 2.4±0.2 | 2.6±0.2 | 2.5±0.2 | 2.6±0.4 | 2.2±0.3 | |

**Table 6a: Cr concentration (mg/kg) in *Eucalyptus* wood grown in soil amended with fly ash and under different treatments**

| Fly ash (%) | $T_1$ (control) | | | $T_2$ (microbes) | | | $T_3$ (soil conditioner) | | | $T_4$ (microbes+SC) | | | $T_5$ (fertilizer) | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | $R_1$ | $R_2$ | $R_3$ | $R_1$ | $R_2$ | $R_3$ | $R_1$ | $R_2$ | $R_3$ | $R_1$ | $R_2$ | $R_3$ | $R_1$ | $R_2$ | $R_3$ |
| $F_0$ (control) | 6.6 | 4.1 | 6.1 | 4.9 | 5.2 | 7.0 | 5.2 | 6.2 | 8.8 | 5.7 | 6.8 | 5.8 | 4.7 | 5.1 | 7.1 |
| $F_1$ (6%) | 6.0 | 8.0 | 9.0 | 9.3 | 9.2 | 8.2 | 9.3 | 7.6 | 9.4 | 8.1 | 7.6 | 10.9 | 7.1 | 10.2 | 10.2 |
| $F_2$ (12%) | 15.0 | 9.2 | 14.5 | 17.8 | 10.4 | 16.8 | 15.6 | 10.5 | 15.7 | 12.5 | 10.8 | 13.6 | 10.6 | 11.0 | 14.5 |
| $F_3$ (18%) | 14.6 | 18.2 | 16.8 | 15.4 | 15.8 | 16.6 | 14.2 | 19.0 | 15.8 | 17.8 | 17.2 | 17.2 | 16.8 | 18.8 | 17.2 |
| $F_4$ (24%) | 18.7 | 15.4 | 15.9 | 17.7 | 16.0 | 15.6 | 16.4 | 16.8 | 18.0 | 16.0 | 14.4 | 15.6 | 17.4 | 15.9 | 17.2 |

**Table 6b: Mean Cr concentration ± SE**

| Fly ash (%) | $T_1$ (control) | $T_2$ (microbes) | $T_3$ (soil conditioner) | $T_4$ (microbes+SC) | $T_5$ (fertilizer) | M±S.E. |
|---|---|---|---|---|---|---|
| $F_0$ (control) | 5.6±0.8 | 5.7±0.6 | 6.8±1.1 | 6.1±0.3 | 15.6±0.7 | 5.9±0.2 |
| $F_1$ (6%) | 7.7±0.9 | 8.9±0.4 | 8.8±0.6 | 8.8±1.0 | 9.2±1.0 | 8.7±0.2 |
| $F_2$ (12%) | 12.9±1.9 | 15.0±2.3 | 13.9±1.7 | 12.3±0.8 | 12.0±1.2 | 13.2±0.6 |
| $F_3$ (18%) | 16.6±1.0 | 15.9±0.4 | 16.3±1.4 | 17.4±0.2 | 17.6±0.6 | 16.8±0.3 |
| $F_4$ (24%) | 16.6±1.0 | 16.4±0.6 | 17.1±0.5 | 15.3±0.4 | 16.8±0.5 | 16.5±0.3 |
| M±S.E. | 11.9±2.3 | 12.4±2.2 | 12.6±2.0 | 12.0±2.1 | 12.2±2.3 | |

**Table 7a: Fe concentration (mg/kg) in *Eucalyptus* wood grown in soil amended with fly ash and under different treatments**

| Fly ash (%) | $T_1$ (control) | | | $T_2$ (microbes) | | | $T_3$ (soil conditioner) | | | $T_4$ (microbes+SC) | | | $T_5$ (fertilizer) | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | $R_1$ | $R_2$ | $R_3$ | $R_1$ | $R_2$ | $R_3$ | $R_1$ | $R_2$ | $R_3$ | $R_1$ | $R_2$ | $R_3$ | $R_1$ | $R_2$ | $R_3$ |
| $F_0$ (control) | 185.0 | 220.0 | 245.0 | 245.0 | 170.0 | 205.0 | 130.0 | 185.0 | 155.5 | 170.0 | 170.0 | 161.0 | 210.0 | 130.0 | 151.5 |
| $F_1$ (6%) | 170.0 | 260.0 | 107.5 | 144.0 | 185.0 | 82.0 | 230.0 | 280.0 | 160.3 | 175.0 | 120.0 | 227.0 | 165.0 | 254.5 | 247.0 |
| $F_2$ (12%) | 228.0 | 138.5 | 270.0 | 322.0 | 108.5 | 255.0 | 168.0 | 129.5 | 245.0 | 169.5 | 204.0 | 184.0 | 127.5 | 166.5 | 175.0 |
| $F_3$ (18%) | 235.0 | 220.0 | 202.0 | 175.5 | 220.0 | 290.0 | 190.0 | 275.0 | 165.0 | 310.0 | 230.0 | 205.0 | 230.0 | 255.0 | 186.0 |
| $F_4$ (24%) | 207.5 | 170.0 | 112.0 | 180.0 | 110.5 | 133.0 | 161.5 | 79.5 | 114.5 | 135.0 | 155.0 | 182.5 | 110.0 | 135.0 | 93.5 |

**Table 7b: Mean Fe concentration ± SE**

| Fly ash (%) | $T_1$ (control) | $T_2$ (microbes) | $T_3$ (soil conditioner) | $T_4$ (microbes+SC) | $T_5$ (fertilizer) | M±S.E. |
|---|---|---|---|---|---|---|
| $F_0$ (control) | 216.7±17.4 | 206.7±21.7 | 156.8±15.9 | 165.5±4.5 | 163.8±23.9 | 181.9±12.3 |
| $F_1$ (6%) | 179.2±44.3 | 137.0±30.0 | 223.4±34.8 | 174.0±30.9 | 222.2±28.7 | 187.2±16.2 |
| $F_2$ (12%) | 212.2±38.8 | 228.5±63.1 | 180.8±34.0 | 185.8±10.0 | 156.3±14.6 | 192.7±12.6 |
| $F_3$ (18%) | 219.0±9.6 | 228.5±33.4 | 210.0±33.3 | 248.3±31.7 | 223.7±20.2 | 225.9±6.4 |
| $F_4$ (24%) | 163.2±27.8 | 141.2±20.5 | 118.5±24.2 | 157.5±13.8 | 112.8±12.1 | 138.6±10.1 |
| M±S.E. | 198.0±11.3 | 188.4±20.5 | 177.9±18.8 | 186.2±16.2 | 175.8±21.1 | |

**Table 8a: Ni concentration (mg/kg) in *Eucalyptus* wood grown in soil amended with fly ash and under different treatments**

| Fly ash (%) | $T_1$ (control) | | | $T_2$ (microbes) | | | $T_3$ (soil conditioner) | | | $T_4$ (microbes+SC) | | | $T_5$ (fertilizer) | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | $R_1$ | $R_2$ | $R_3$ | $R_1$ | $R_2$ | $R_3$ | $R_1$ | $R_2$ | $R_3$ | $R_1$ | $R_2$ | $R_3$ | $R_1$ | $R_2$ | $R_3$ |
| $F_0$ (control) | 0.2 | 2.4 | 2.9 | F | 0.8 | 2.4 | 0.3 | 0.6 | 2.9 | 1.4 | 2.4 | 4.1 | 1.5 | 1.3 | 2.7 |
| $F_1$ (6%) | 3.5 | 3.2 | 3.0 | 4.4 | 2.6 | 4.5 | 2.6 | 4.1 | 4.8 | 2.6 | 3.2 | 4.8 | 3.0 | 2.4 | 6.0 |
| $F_2$ (12%) | 2.6 | 4.4 | 4.7 | 4.0 | 5.4 | 7.9 | 4.9 | 6.6 | 6.0 | 4.6 | 4.4 | 8.5 | 4.5 | 4.6 | 6.4 |
| $F_3$ (18%) | 6.2 | 4.8 | 9.1 | 9.4 | 8.5 | 6.2 | 6.6 | 8.1 | 8.6 | 7.5 | 9.6 | 8.7 | 10.8 | 7.8 | 6.8 |
| $F_4$ (24%) | 14.0 | 15.0 | 14.0 | 16.6 | 14.0 | 13.6 | 13.2 | 15.1 | 13.8 | 16.4 | F | 14.2 | 16.0 | 13.9 | 13.4 |

**Table 8b: Mean Ni concentration ± SE**

| Fly ash (%) | $T_1$ (control) | $T_2$ (microbes) | $T_3$ (soil conditioner) | $T_4$ (microbes+SC) | $T_5$ (fertilizer) | M±S.E. |
|---|---|---|---|---|---|---|
| $F_0$ (control) | 1.8±0.8 | 1.6±0.8 | 1.3±0.8 | 2.6±0.8 | 1.8±0.4 | 1.8±0.2 |
| $F_1$ (6%) | 3.2±0.2 | 4.1±0.8 | 3.8±0.6 | 3.5±0.6 | 3.8±1.1 | 3.7±0.2 |
| $F_2$ (12%) | 3.9±0.6 | 5.8±1.1 | 5.8±0.5 | 5.8±1.3 | 5.2±0.6 | 5.3±0.4 |
| $F_3$ (18%) | 6.7±1.3 | 8.0±1.0 | 7.8±0.6 | 8.6±0.6 | 8.5±1.2 | 7.9±0.3 |
| $F_4$ (24%) | 14.4±0.3 | 14.7±1.0 | 14.0±0.6 | 15.3±1.1 | 14.4±0.8 | 14.5±0.2 |
| M±S.E. | 6.0±2.2 | 6.8±2.2 | 6.6±2.12 | 7.2±2.2 | 6.7±2.0 | |

**Table 9a: Pb concentration (mg/kg) in *Eucalyptus* wood grown in soil amended with fly ash and under different treatments.**

| Fly ash (%) | $T_1$ (control) | | | $T_2$ (microbes) | | | $T_3$ (soil conditioner) | | | $T_4$ (microbes+SC) | | | $T_5$ (fertilizer) | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | $R_1$ | $R_2$ | $R_3$ | $R_1$ | $R_2$ | $R_3$ | $R_1$ | $R_2$ | $R_3$ | $R_1$ | $R_2$ | $R_3$ | $R_1$ | $R_2$ | $R_3$ |
| $F_0$ (control) | 7.6 | 8.1 | F | 1.9 | 2.2 | 13.8 | 2.9 | 4.1 | 4.1 | 3.8 | 2.6 | 8.3 | 9.6 | 6.9 | 9.8 |
| $F_1$ (6%) | 11.0 | 8.8 | 5.2 | 11.1 | 10.4 | 11.7 | 14.6 | 8.4 | 12.9 | 5.6 | 7.4 | 17.9 | 10.4 | 9.7 | 9.2 |
| $F_2$ (12%) | 16.8 | 11.2 | 16.0 | 9.2 | 11.3 | 16.3 | 14.6 | 13.0 | 14.7 | 16.2 | 10.5 | 14.6 | 14.0 | 23.2 | 16.9 |
| $F_3$ (18%) | 20.5 | 17.3 | 22.0 | 19.1 | 19.2 | 22.0 | 16.0 | 15.4 | 22.8 | 20.5 | 19.3 | 23.6 | 23.4 | 14.2 | 13.9 |
| $F_4$ (24%) | 15.8 | 15.4 | 13.5 | 13.4 | 18.8 | 15.2 | 10.8 | 15.2 | 16.0 | 13.2 | 17.4 | 19.2 | 15.4 | 11.0 | 19.4 |

**Table 9b: Mean Pb concentration ± SE**

| Fly ash (%) | $T_1$ (control) | $T_2$ (microbes) | $T_3$ (soil conditioner) | $T_4$ (microbes+SC) | $T_5$ (fertilizer) | M±S.E. |
|---|---|---|---|---|---|---|
| $F_0$ (control) | 7.9±0.2 | 6.0±3.9 | 3.7±0.4 | 4.9±1.7 | 8.8±1.0 | 6.2±0.9 |
| $F_1$ (6%) | 8.3±1.7 | 11.0±0.4 | 12.0±1.9 | 10.3±3.8 | 9.8±0.3 | 10.3±0.6 |
| $F_2$ (12%) | 14.7±1.7 | 12.3±2.1 | 14.1±0.6 | 13.8±1.7 | 18.0±2.7 | 14.6±0.9 |
| $F_3$ (18%) | 19.9±1.4 | 20.1±0.9 | 18.1±2.4 | 21.1±1.3 | 17.3±3.0 | 19.3±0.7 |
| $F_4$ (24%) | 14.9±0.7 | 15.8±16.0 | 14.0±1.64 | 16.6±1.8 | 15.3±2.4 | 15.3±0.4 |
| M±S.E. | 13.1±2.3 | 13.1±2.3 | 12.4±2.4 | 13.3±2.8 | 13.8±1.9 | |

## Table 10: Analysis of variance

| | | Cellulose | Hemicelluloses | Lignin | Ash | Cu | Ni | Cr | Pb | Fe |
|---|---|---|---|---|---|---|---|---|---|---|
| **Fly Ash** | SS | 761.2 | 4.09 | 433.66 | 0.82 | 14.44 | 14441.7 | 1375.87 | 1498.21 | 58416.51 |
| | df | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 |
| | MS | 190.3 | 1.02 | 108.42 | 0.2 | 3.61 | 360.42 | 343.97 | 374.55 | 14604.13 |
| | F | $5.49^{***}$ | 0.51 | $6.08^{***}$ | 0.41 | $5.66^{***}$ | $183.61^{***}$ | $110.84^{***}$ | $33.4^{***}$ | $6.03^{***}$ |
| | % V | 25.42 | 3.62 | 28.12 | 2.19 | 27.42 | 90.92 | 87.77 | 65.11 | 26.28 |
| **Treatment** | SS | 66.14 | 1.20 | 14.31 | 0.74 | 1.38 | 10.54 | 4.86 | 16.42 | 4776.14 |
| | df | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 |
| | MS | 16.53 | 0.3 | 3.58 | 0.19 | 0.34 | 2.64 | 1.21 | 4.1 | 1194.03 |
| | F | 0.476 | 0.15 | 0.2 | 0.37 | 0.54 | 1.34 | 0.39 | 0.37 | 0.49 |
| | % V | 2.21 | 1.06 | 0.88 | 1.99 | 2.63 | 0.73 | 0.31 | 0.74 | 2.14 |
| **Interaction** | SS | 432.95 | 8.26 | 197.74 | 10.66 | 4.93 | 10.16 | 31.67 | 156.39 | 38167.34 |
| | df | 16 | 16 | 16 | 16 | 16 | 16 | 16 | 16 | 16 |
| | MS | 27.06 | 0.52 | 12.36 | 0.67 | 0.31 | 0.64 | 1.98 | 9.77 | 2385.46 |
| | F | 0.78 | 0.26 | 0.69 | 1.32 | 0.48 | 0.32 | 0.64 | 0.87 | 0.98 |
| | % V | 14.46 | 7.3 | 12.97 | 28.47 | 9.37 | 0.72 | 2.02 | 6.92 | 17.08 |
| **Error** | SS | 1734.37 | 99.62 | 892.17 | 25.21 | 31.91 | 98.19 | 155.17 | 559.15 | 121173.72 |
| | df | 50 | 50 | 50 | 50 | 50 | 50 | 50 | 50 | 50 |
| | MS | 34.69 | 2 | 17.48 | 0.5 | 0.64 | 1.96 | 3.1 | 11.18 | 2423.47 |

* significant at $p < 0.05$
** significant at $P < 0.01$
*** significant at $P < 0.001$

**Table 11a: The Least Significant Difference (LSD) Test**

| Fly ash con. | Cellulose<br>LSD 0.05= 4..31 | Lignin<br>LSD 0.05=3.09 |
|---|---|---|
| $F_0$ (0%) | 41.7 ab | 31.3 c |
| $F_1$ (6%) | 40.8 b | 35.6 ab |
| $F_2$ (12%) | 45.4a | 32.8 bc |
| $F_3$ (18%) | 38.6bc | 38.1a |
| $F_4$ (24%) | 35.9c | 32.9 bc |

The rows having a common letter are not significant at $P < 0.05$.

**Table 11b: The Least Significant Difference (LSD) Test**

| Fly ash con. | Cu<br>LSD 0.05=0.59 | Cr<br>LSD 0.05=1.29 | Fe<br>LSD 0.05=36.1 | Ni<br>LSD 0.05=1.03 | Pb<br>LSD 0.05=2.45 |
|---|---|---|---|---|---|
| $F_0$ (0%) | 2.2 bc | 5.9 d | 181.9 b | 1.8 e | 6.2 d |
| $F_1$ (6%) | 2.0 c | 8.7 c | 187.2 b | 3.7 d | 10.3 c |
| $F_2$ (12%) | 2.0 c | 13.2 b | 192.7 ab | 5.3 c | 14.6 b |
| $F_3$ (18%) | 3.2 a | 16.8 a | 225.9 a | 7.9 b | 19.3 a |
| $F_4$ (24%) | 2.7 ab | 16.5 a | 138.6 c | 14.5 a | 15.3 b |

The rows having a common letter are not significant at $P < 0.05$.