Degradation Efficacy of *Pinus radiata* Don Needle Leaf, Twig and Bark by Wood Degrading Fungi in Forest Ecosystem

M. N. Abubacker and M. Prince

Abstract The degradation of lignin, holocellulose and hot water soluble content of *Pinus radiata* needle leaf, twig and bark by wood degrading fungi was studied in virgin forest ecosystem of Doddabetta, Nilgiris for a period of 180 days. The study revealed that the maximum percentage of lignin degradation of needle leaf material with *Heterobasidium annosum* was 8.8, twig with the same fungus was 18.3, and the bark with 19.5. For holocellulose needle leaf degradation with a maximum of 10.3 for *Thelopora terrestris* fungus for twig with the same fungi shows 12.1 and for bark *Polyporus squamosus* with 16.9 of degradation. Hot water soluble content was maximum of 18.7% with *Colricia perennis* fungus for the needle leaf, for twig 10.5% for the same fungi and for bark with 9.0% for *Polyporus squamosus* for a period of 180 days. The elemental status of mixed samples of needle leaf, twig and bark constitute the forest litter of *P. radiata* were inoculated with *H. annosum*, *T. terrestris*, *P. squamosus* and *C. perennis* mycelia. The SEM–EDX analysis revealed the maximum availability of elements at 180 days of degradation when compared with initial stage.

Keywords Degradation · Holocellulose · Lignin · Hot water soluble content · SEM–EDX

1 Introduction

Forests represent approximately 27% of the world's land area and wood is the predominant, commercial product from forests. Wood is used for the manufacture of wood-based products. Logging operations in forests usually generate abundant amounts of waste such as residual wood, branches, twigs, leaves and bark. The

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waste amounts for more than 60% of the total biomass (Kuhad et al. 1997). Forest is the suitable-substratum where the micro and macro fungi not only live but also to degrade the leaf litter fallen from the trees and supply most valuable nutrients back to the forest soil. A large amount of plant waste (leaf litter) is being continuously accumulated on the forest soil. A part of the waste gets into the soil and an intensive degradation process starts. Intensity of this decay process depends on different environmental factors such as: species of plant, sort of soil, moisture, temperature and microbial associations. In order to increase the efficiency of these metabolic processes, the scientific society is making strong efforts in this area. Moreover, the aim of the latest researches are oriented to intensify plant remnants decay and modify metabolites enriching the soil by useful biologically active substances and finding materials able to enrich forage and food by important biologically valuable additions (Kelley 1992; Varnaite 2001; Abubacker and Prince 2013, 2015).

1.1 Forest Biomass

The composition of lignocellulose materials forms the wood wall structure. The major component of lignocellulose material is cellulose, along with lignin and hemicellulose. Cellulose and hemicellulose are macromolecules formed from different sugars; whereas lignin is an aromatic polymer synthesized from phenylpropanoid precursors. The composition and percentages of these polymers vary from one plant species to another. Moreover, the composition within a single plant varies with age, stage of growth, and other conditions (Jeffries 1994; Krishna and Mohan 2017).

Hemicellulose is a complex carbohydrate polymer and makes up 25–30% of total dry weight of wood. It is a polysaccharide with a lower molecular weight than cellulose. It consists of D-xylose, D-mannose, D-galactose, D-glucose, L-arabinose, 4-O-methyl-glucuronic, D-galacturonic and D-glucuronic acids. Sugars are linked together by β-1,4- and occasionally β-1,3-glycosidic bonds. The principal component of hardwood hemicellulose is glucuronoxylan whereas glucomannan is predominant in softwood. Structures of hemicelluloses are described in several reviews the main difference with cellulose is that hemicellulose has branches with short lateral chains consisting of different sugars. In contrast to cellulose, they are easily hydrolyzable polymers. They do not form aggregates, even when they are co-crystallized with cellulose chains (Jeffries 1994).

Lignin (along with cellulose) is the most abundant polymer in nature. It is present in the cellular cell wall, conferring structural support, impermeability, and resistance against microbial attack and oxidative stress. Structurally, lignin is an amorphous heteropolymer, non-water soluble and optically inactive; it consists of phenylpropane units joined together by different types of linkages (Jeffries 1994).

1.2 Decomposition

Litter decomposition is a fundamental process to ecosystem, responsible of carbon and nutrient cycling. The chemical composition and the structural characteristics of litter determines the "decomposability" resulting in fast or slow degradation and the fraction of recalcitrant residue that determine the buildup of soil organic matter (Berg and McClaugherty 2008; Baldrin and Lindahi 2017). Decomposition process of leaf litter is generally divided into early and late stages with different dominant organic chemical components limiting the decomposition rate. In the early stages of decomposition soluble components decay or are leached away very rapidly; cellulose and hemicellulose decompose faster in nutrient rich versus nutrient poor litter; thus the length of the early stage may range from a few months to more than a year. As litter decomposes, concentrations of lignin and nitrogen (N) increase (Berg et al. 1997). Litters with low initial nitrogen concentration may accumulate more nitrogen than those with a high level (Hobbie and Vitousek 2000; Pirjo Koivusarri Mysore et al. 2019). Site factors also contribute to nitrogen immobilization into decomposing litter and nitrogen accumulation in litter may be enhanced by soil N levels (Virzo De Santo et al. 1998; Sun et al. 2020). Fungi are the best-known microorganisms capable of degrading these three polymers. The substrates being insoluble and fungal degradation have to occur exocellularly, either in association with the outer cell envelope layer or extracellularly.

The present work is focused on the degradation efficacy of *Pinus radiata* Don, needle leaf, twig and bark by wood degrading fungi in forest ecosystem. The name of the Fungi are *Colricia perennis*, *Heterobasidium annosum*, *Polyporus squamosus, Thelophora terrestris.*

2 Materials and Methods

2.1 Study Area

The area of the study for this research work was identified based on the undisturbed virgin forest ecosystem of Doddabetta, Nilgiris, Tamil Nadu, India, which is 86 km from Coimbatore situated at 11°24′08.7° N and 76°44′12.2° E with a elevation of 8652 ft (2637 m) and 9 km from Ooty town (Satellite Images-Fig. 1). The area surrounding Doddabetta is mostly forest, Sholas cover the hollows of its slopes, which receives both South-west and North-east Monsoon rains. The average South-west Monsoon rain fall 900–1050 mm, the average North-east monsoon rain fall 350–450 mm. The present research work was carried out with the Gymnosperm forest *Pinus radiata* Don. of Doddabetta, Nilgiris, India (Fig. 1).

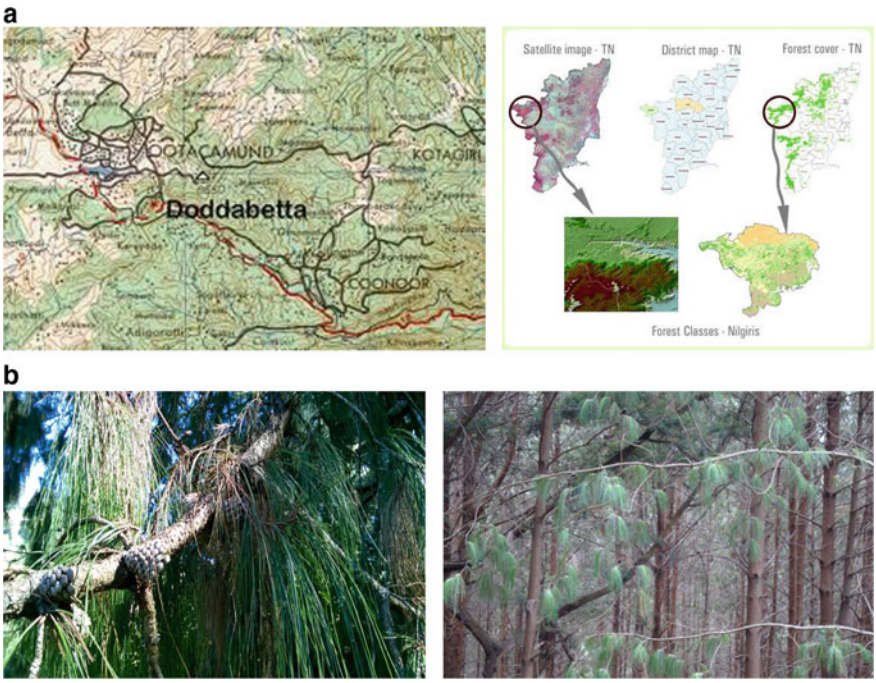

Fig. 1 **a** Study area. **b** Pine forest *with Pinus radiata* Don

2.2 Decomposition Study by Litterbag Method

The leaf, twig and bark used for decomposition study was picked from the respective forest floor and placed in sterile polythene bags. The fungal fruit body was also collected in sterile polythene bags and brought to the laboratory and inoculated in the malt-extract agar medium and the growth mycelia was used for further experimental work. They were cultured in medium modified by Miura and Kudo (1970) contains glucose 0.1%, KH_2PO_4 0.1%, $MgSO_4$, $7H_2O$ 0.02%, KCl 0.02%, $NaNO_3$ 0.2%, Yeast extract 0.02%. The decomposition study pertaining to holocellulose, lignin and hot water soluble content was studied with a litter bag methods (Crossley and Hoglund 1962) as well as Erlenmeyer flask method.

2.3 Determination of Lignin Content

TAPPI Standard method, T 222 OS-74 (2009) procedure was followed for the determination of lignin content. One gram of air-dried sample was weighed out accurately in a weighing bottle and transferred to a 50 ml beaker, then 10 ml of 72% sulphuric acid was added carefully with a pipette and the mixture was stirred with a glass rod.

The mixture was moved quantitatively with a wash bottle of a 500 ml round-bottle flask and diluted with water until the final volume was 300 ml. The solution was then refluxed for 3 h, filtered in a glass filter and dried in an oven at 105 °C for 12 h. The crucible was cooled in a desiccator for 15 min and then weighed accurately. The glass filter containing the lignin was reported as a percentage by weight of the dried sample. Lignin content was calculated using

$$\text{Lignin content}(\%) = \frac{\text{Oven dried weight of lignin}}{\text{Oven dried weight of initial sample}} \times 100 \quad (1)$$

2.4 Determination of Holocellulose Content

Holocellulose content was determined with reference to ASTMD 1104-56, (1978) method, in which 1 g of sample was placed in an Erlenmeyer flask (250 ml) and 150 ml of distilled water was added. While slowly shaking, 1 g of $NaClO_2$ and 0.2 ml of acetic acid were added and the flask was covered with glass and boiled at 70–80 °C for 60 min. Again, 1 g of $NaClO_2$ and 0.2 ml of acetic acid were added and boiled. After cooling, the sample was filtered using a filter flask and washed with hot water until free of acid. Afterward, the insoluble portion was dried in an oven at 105°c for 4 h, cooled in a desiccator and weighed repeatedly until obtaining a constant weight. Holocellulose content was calculated as follows.

$$\text{Holocellulose content}(\%) = \frac{\text{Oven dried weight of holocellulose}}{\text{Oven dried weight of initial sample}} \times 100 \quad (2)$$

The soluble of treated leaf, twig and bark samples were examined with reference to ASTMD1110-87 (2007). Two-gram sample was oven-dried and placed into a 250 ml Erlenmeyer flask containing 200 ml of distilled water. A reflux condenser was attached to the flask and the apparatus was placed in a gently boiling water bath for three hours with constant shaking. Special attention was given to insure that the level of the solution in the flask remained below that of the boiling water. Samples were then removed from the water bath and filtered by vacuum suction into a glass filter of known weight. The residue was washed with hot water before the glass-filter was oven-dried at 103 ± 2 °C. The glass-filter was then cooled in a desiccator and weighed until a constant weight was obtained. The following formula was used to obtain the hot water soluble of the sample

$$\text{Hotwatersoluble}(\%) = \frac{W_1}{W_1 - W_2} \times 100 \quad (3)$$

where

W_1 weight of oven – dry test sample (g).
W_2 Weight of oven – dry sample after extraction with hot water (g).

2.5 SEM–EDX Elemental Analysis

Scanning Electron Microscopy equipped with Energy Dispersive X-ray (SEM–EDX) analysis was performed to determine the cellular and sub-cellular structure of degrading biomass and the elemental levels at different degradation stages (Vinod and Sashidhar 2010). The leaf, twig and bark mixed degraded samples were dried and ground into fine powder and then placed in the steel stab with carbon tape and sputter coated with gold particle for 50 s in high vacuum conditions. Elemental analysis and Scanning Electron Microscopic images coupled with energy dispersive X-ray consisting 3.5 and 2.5 nm resolution for tungsten filament and LaB6 and EDX detector resolution 133 eV of the degraded samples was assessed.

3 Results and Discussion

Degradation of lignin, holocellulose, hot water soluble content of *Pinus radiata* Don needle leaf, twig and bark by wood degrading fungi. The degradation abilities of lignin, holocellulose, hot water soluble content of *Pinus radiata* needle leaf, twig and bark by specific wood degrading fungi of *P. radiata* forest ecosystem was presented in (Table 1).

The lignin content of needle leaf as control 23.8% at 90 days, the treated samples with fungi showed the decrease in the lignin content 17.1–19.5% and after 180 days it was 13.5–15.3%, depending on the fungal genera (Table 1).

The most efficient lignin degrading fungi of needle leaf was found to be *Colricia perennis* with 6.7% at 90 days and 8.8% at 180 days of degradation.

For twig, the initial lignin content of control was 52.0% which is decreased to 38.0–44.2% at 90 days treated sample with fungi and at 180 days it was 50.8% for control, was decreased from 32.5 to 38.0% depending on the fungal genera (Table 1). The most efficient lignin degrading fungi for twig was *Heterobasidion annosum* with 14.0% at 90 days and 18.7% at 180 days of degradation.

In the case of bark, the control sample have shown 90.2% at 90 days, the treated samples with fungi showed the decrease in the lignin control, i.e., 68.0–82.0% and at 180 days it was 66.5 to 78.3% as against the control 88.0%. The most efficient lignin degrading fungi of bark was found to be 15.6% for *Heterobasidion annosum* at 90 days, but at 180 days it was found to be 11.5% for *Colricia perennis* (Figs. 2, 3, 4, 5 and 6).

Table 1 Degradation of lignin, holocellulose and hot water soluble content of *Pinus radiata* Don needle leaf, twig and bark by wood degrading fungi

Name of the fungi		Leaf				Twig				Bark			
		90 days	%	180 days	%	90 days	%	180 days	%	90 days	%	180 days	%
Colricia perennis	LC	17.1 ± 0.44	6.7	14.9 ± 0.40	7.4	39.4 ± 0.34	12.6	37.8 ± 0.36	13.0	80.4 ± 0.65	10.2	66.5 ± 0.50	21.5
	HC	16.1 ± 0.44	5.1	15.0 ± 0.15	5.5	41.3 ± 0.08	3.6	35.3 ± 0.39	9.3	68.0 ± 0.20	4.8	59.5 ± 0.45	12.5
	HWSC	36.0 ± 0.50	5.7	72.5 ± 0.06	18.7	64.2 ± 0.32	9.9	84.0 ± 0.25	10.5	68.5 ± 0.55	4.2	81.5 ± 0.40	6.5
Heterobasidion annosum	LC	18.2 ± 0.14	5.6	13.5 ± 1.00	8.8	38.0 ± 0.60	14.0	32.5 ± 0.10	18.3	74.6 ± 0.40	15.6	68.5 ± 0.45	19.5
	HC	18.0 ± 0.30	3.2	12.5 ± 0.51	8.0	40.0 ± 0.40	4.9	36.2 ± 0.38	8.4	60.0 ± 0.50	12.8	58.0 ± 0.65	14.0
	HWSC	31.5 ± 0.50	1.2	62.3 ± 0.10	8.5	68.2 ± 0.41	13.9	82.0 ± 0.30	8.5	67.1 ± 0.40	2.8	80.1 ± 0.53	5.1
Polyporus squamosus	LC	18.5 ± 0.20	5.3	15.0 ± 0.21	7.3	40.2 ± 0.18	11.8	36.0 ± 0.10	14.8	81.8 ± 0.08	8.4	70.0 ± 0.18	18.0
	HC	17.6 ± 0.10	3.6	14.5 ± 0.40	6.0	42.5 ± 0.20	2.4	39.0 ± 0.12	5.6	61.0 ± 0.20	11.8	55.1 ± 0.02	16.9
	HWSC	34.0 ± 0.38	3.7	62.5 ± 0.40	8.7	68.0 ± 0.48	13.7	77.0 ± 0.15	3.5	67.8 ± 0.40	3.5	84.0 ± 0.41	9.0
Thelophora terrestris	LC	19.5 ± 0.30	4.3	15.3 ± 0.40	7.0	44.2 ± 0.40	7.8	38.0 ± 0.10	12.8	82.0 ± 0.40	8.2	78.3 ± 0.17	9.7
	HC	17.5 ± 0.45	3.7	10.2 ± 0.40	10.3	39.0 ± 0.10	5.9	32.5 ± 0.30	12.1	67.3 ± 0.20	5.5	60.0 ± 0.38	12.0
	HWSC	32.5 ± 0.40	2.2	71.5 ± 0.33	17.7	62.1 ± 0.42	7.8	78.9 ± 0.65	5.4	68.0 ± 0.65	3.7	78.5 ± 0.15	3.5
Control	LC	23.8 ± 0.38	–	22.3 ± 1.10	–	52.0 ± 0.10	–	50.8 ± 0.58	–	90.2 ± 0.18	–	88.0 ± 0.08	–
	HC	21.2 ± 0.10	–	20.5 ± 1.15	–	44.9 ± 0.39	–	44.6 ± 0.50	–	72.8 ± 0.40	–	72.0 ± 1.00	–
	HWSC	30.3 ± 0.47	–	53.8 ± 0.45	–	54.3 ± 0.38	–	73.5 ± 0.10	–	64.3 ± 0.18	–	75.0 ± 0.36	–

LC Lignin content, *HC* Holo Cellulose, *HWSC* Hot water soluble content

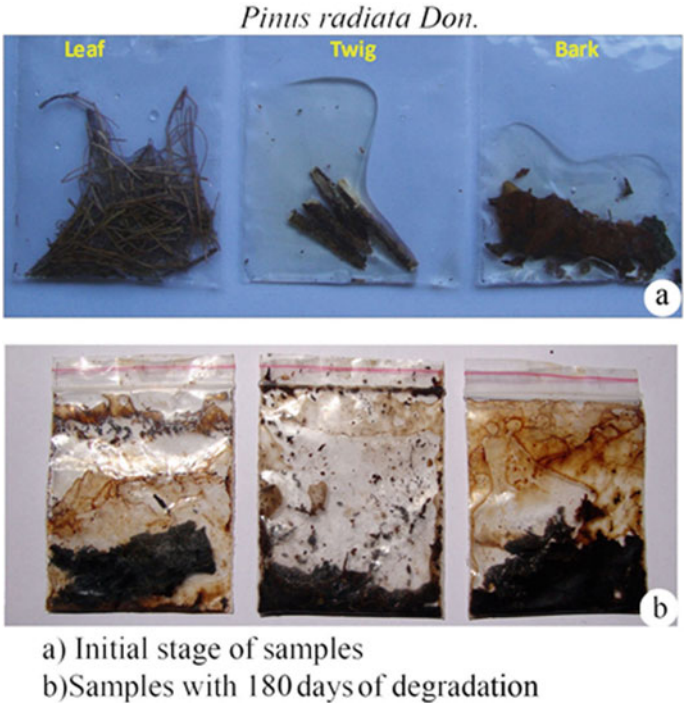

Fig. 2 Bio degradation samples of Pinus radiata Don

3.1 Degradation of Lignin

Lignocellulose is the predominant component of woody plant and dead plant materials, and the most abundant biomass on earth. Lignin and holocellulose in the biomass structure are the major energy sources available to decomposer organisms constituting 70–80% of fresh organic material (Swift et al. 1979). Lignin is a recalcitrant plant polymer and its mineralization by white rot basidiomycetes plays a major role in carbon recycling (Martinez et al. 2005). White rot fungi are wood degrading organisms capable of decomposing all wood polymers, lignin, cellulose and hemicelluloses (Hakala 2007). Holocellulose, a polysaccharide containing cellulose and hemicellulose (Petterson 1984) is a major component of wood suitable-for fungal growth. Polysaccharide content generally ranges between 60 and 80% (w/w) in hardwood (Willfor et al. 2005). However, decomposition rate of cellulose is higher than that of lignin (Fioretto et al. 2005). White-rot fungi belong to the basidiomycetes and their activity is usually related to the moisture content of wood (Blanchette 1995). The decaying fungi belong to saprophyte fungal organisms, since they live on dead or residual vegetation, decomposing them into simpler molecular compounds (Dubeux et al. 2006; Ohkuma et al. 2001).

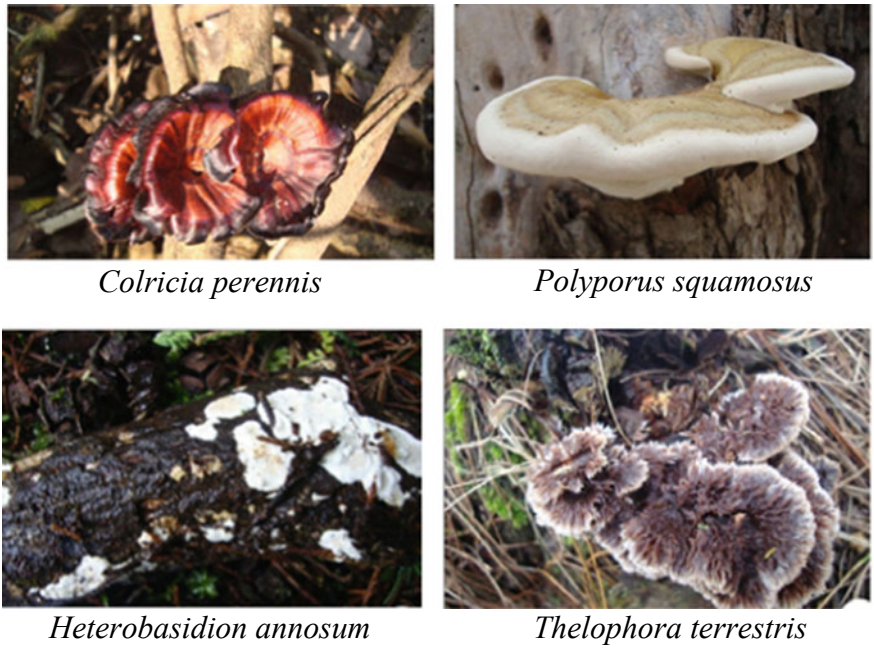

Fig. 3 Wood Degrading fungi source—*Pinus radiata* Don

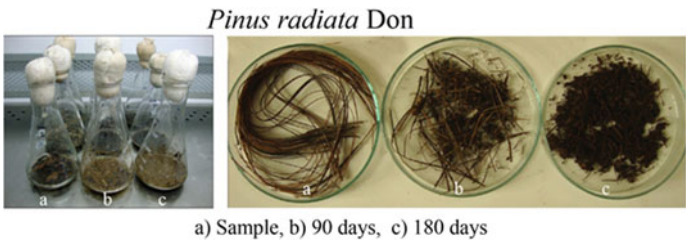

Fig. 4 Biodegradation of leaf samples

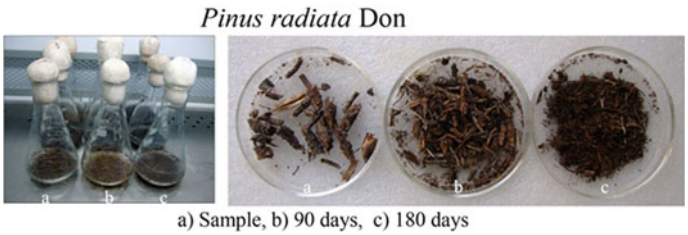

Fig. 5 Biodegradation of twig samples

Fig. 6 Biodegradation of bark samples

Fungi require a carbon source, macronutrients such as nitrogen, phosphorous and potassium and certain trace elements for their growth. Carbon serves primarily as an energy source for the microorganisms, while a small fraction of the carbon in incorporated into their cells (Tuomela et al. 2000). Biomass including leaf, twigs, bark and other residual materials of forest ecosystem, naturally undergo degradation due to fungal enzymatic action. This causes increase in CO_2 in the environment. Therefore, it would be better for the woody materials to be recycled by biological degradation or removal of lignin (Watanabe et al. 2003). It was found that lignin content of either leaf, twigs or bark degraded effectively by naturally occurring lignin degrading fungi of forest ecosystem. The present work revealed that in *Pinus radiata* forest ecosystem consists of specific lignin degrading fungi are *Colricia perennis, Heterobasidion annosum, Polyporus squamosus* and *Thelophora terrestris* in which the most efficient lignin degrading fungi of needle leaf was *Colricia perennis* with 6.7 and 8.8% of degradation for 90th day and 180th days respectively. For twig *Heterobasidion annosum* with 14.0% at 90 days and 18.3% at 180 days of degradation. In the case of bark *H. annosum* with 15.6% and *C. perennis* 11.5% of degradation (TAPPI Standard method, T 222 OS-74 (2009)).

3.2 Holocellulose Degradation

The degradation ability of holocellulose of control sample of needle leaf of 90 days was 21.2% and at 180 days it was 20.5%, whereas the fungal treated samples have shown 16.1–18.0% for 90 days and at 180 days it was 10.2–15.0% degrading in the fungal genera involved in the degradation (Table 1). The most efficient holocellulose degrading fungi was *Colricia perennis* with the degradation percentage 5.1 for 90 days of degradation and 10.3% degradation by *Thelophora terrestris* for 180 days of degradation (ASTMD1110-87 (2007)).

The degradation of holocellulose for twig of *Pinus radiata*, control samples have shown 44.9% and 44.6% for 90 days and 180 days respectively, whereas the fungal treated samples have shown 39.0 to 41.3% for 90 days of degradation and 32.5–39.0% for 180 days samples with respect to the fungal genera involved in degradation (Table 1). The most efficient fungi involved in the holocellulose degradation of twig was

found to be *Thelophora terrestris* with 5.9 and 12.1% for both 90 and 180 days of degradation. In the case of degradation of bark the control samples have shown 72.8 and 72.0% for 90 and 180 days. The fungal treated ones have shown 60.0 and 68.0% of degradation for 90 and 55.1 and 60.6% for 180 days, with respect to the fungal genera involved in the holocellulose degradation (Table 1). The most efficient fungi involved in the degradation of bark was found to be *Heterobasidion annosum* with 12.8% for 90 days, whereas *Polyporus squamosus* with 16.9% for 180 days of degradation (Figs. 2, 3, 4, 5 and 6).

The *Pinus radiata* forest ecosystem consists of specific holocellulose degrading fungi are *Colricia perennis, Heterobasidion annosum, Polyporus squamosus* and *Thelophora terrestris* in which the most efficient holocellulose degrading fungi of needle leaf was *Colricia perennis* with 5.1% at 90 days and *Thelophora terrestris* with 10.3% at 180 days. For twig *Thelophora terrestris* with 5.1% at 90 days and 12.1% at 180 days of degradation. In the case of bark *Heterobasidion annosum* with 12.8% and *Polyporus squamosus* with 16.9% of degradation (ASTMD 1104-56 (1978)).

3.3 Hot Water Soluble Content

The volume of the soluble content of *Pinus radiata* needle leaf, twig and bark in hot water is shown in Table 1. It was found that the duration of inoculation in all the sample tested was found to be increased in solubility in hot water treatment. The value of soluble content varied depending on the fungi inoculated. For needle leaf the value of soluble content was 31.5–36.0% as against control sample 30.3% in 90 days. At 180 days, the value of soluble content was 62.3–72.5% as against the control sample 53.8%. The maximum hot water soluble content was recorded in *Colricia perennis,* 5.7% for 90th day of degradation and 18.7% on the 180th day for the same fungus. In the case of twig, the percentage was between 62.1 and 68.2% as against 54.3% for control in 90 days. In 180 days, the results are between 77.0 and 84.0% when compared with the control 73.5%. The maximum hot water soluble content was found in *Heterobasidion annosum* with 13.9% on the 90^{th} day and 10.5% on the 180^{th} day of degradation for *Colricia perennis.* For bark sample, the percentage of soluble content was 67.1–68.5% as against the control, it was 64.3% at 90 days. In 180 days, the results are between 78.5 and 84.0% as against the control it was 75.0%. The maximum hot water soluble content was found in *Colricia perennis* with 4.2% on the 90th day and 9.0% on the 180th day of degradation for *Polyporus squamosus.*

In this study, the hot-water solubility of treated samples increased significantly with incubation time meaning that some amount of lignocellulose content was degraded. This is presumably supported by the monosaccharides in leaf, twig and bark samples like xylose, mannose and glucose (Pinto et al. 2005; Abubacker and Kirthiga 2015) which are soluble in water, besides the degradation of cellulose containing polymers and polysaccharides into simpler components like monomers

through fungal activity (Blanchette et al. 1994). In both 90 and 180 days of incubation, the hot water soluble content of all treated samples increased. Perhaps the soluble matter was consumed for energy by the fungi, since lignin and holocellulose content were less decreased.

3.4 SEM–EDX Elemental Analysis of Mixed Degraded Samples

The SEM–EDX elemental analysis of degradation of mixed samples were conducted for all the four forest materials namely leaf, twig and bark mixed samples. For *Pinus radiata* the degradation sampling was conducted at initial stage, 90th day and 180th day of degradation. In *Pinus radiata* the initial stage the elements available in the mixed samples of biomass shown C 64.2%, O 34.39%, Mg 0.10%, Al 0.23%, Si 0.18%, P 0.13%, S 0.17%, Cl 0.08%, K 0.40% and Fe 0.11%. On the 90th day of degradation the available elements were found to be C 7.95%, O 56.05%, Mg 0.57%, Al 11.35%, Si 12.83%, Cl 0.14%, K 0.67%, Ca 0.24%, Ti 0.44% and Fe 9.76%. On 180th day of degradation the following elements were recorded. C 32.18%, N 2.19%, O 55.26%, Na 0.15%, Mg 0.25%, Al 5.15%, Si 5.08%, P 0.55%, S 0.26%, Cl 0.23%, K 0.53%, Ti 0.10%, Mn 0.14% and Fe 2.31% (Fig. 7).

In *Pinus radiata,* the initial stage the elements available in the mixed samples of biomass shown C 64.2%, O 34.39%, Mg 0.10%, Al 0.23%, Si 0.18%, P 0.13%,S 0.17%, Cl 0.08%, K 0.40% and Fe 0.11%. On the 90th day of degradation the available elements were found to be C 7.95%, O 56.05%, Mg 0.57%, Al 11.35%, Si 12.83%, Cl 0.14%, K 0.67%, Ca 0.24%, Ti 0.44% and Fe 9.76%. On 180th day of degradation the following elements were recorded. C 32.18%, N 2.19%, O 55.26%, Na 0.15%, Mg 0.25%, Al 5.15%, Si 5.08%, P 0.55%, S 0.26%, Cl 0.23%, K 0.53%,Ti 0.10%, Mn 0.14% and Fe 2.31% (Fig. 7).

4 Conclusion

The rate of degradation of *P. radiata* needle leaves, twigs and barks varied depending on the fungal genera inoculated. An increase in incubation time tended reducing both lignin and holocellulose content. However, the reduction rate was not significant, therefore more time is needed to degrade lignin rather than other components in the sample. This report will help to gain the insight of lignin and holocellulose degradation in the early stage in the natural forest ecosystem**.** Fungi play a vital role in plant litter decomposition in forest ecosystem through nutrient cycling and humus formation in soil because they colonize the lignocellulosic matrix in litter that other organisms are unable to decompose (Lodge 1985; Hunt et al. 1989). The present research work with SEM–EDX elemental analysis of degraded mixed samples of

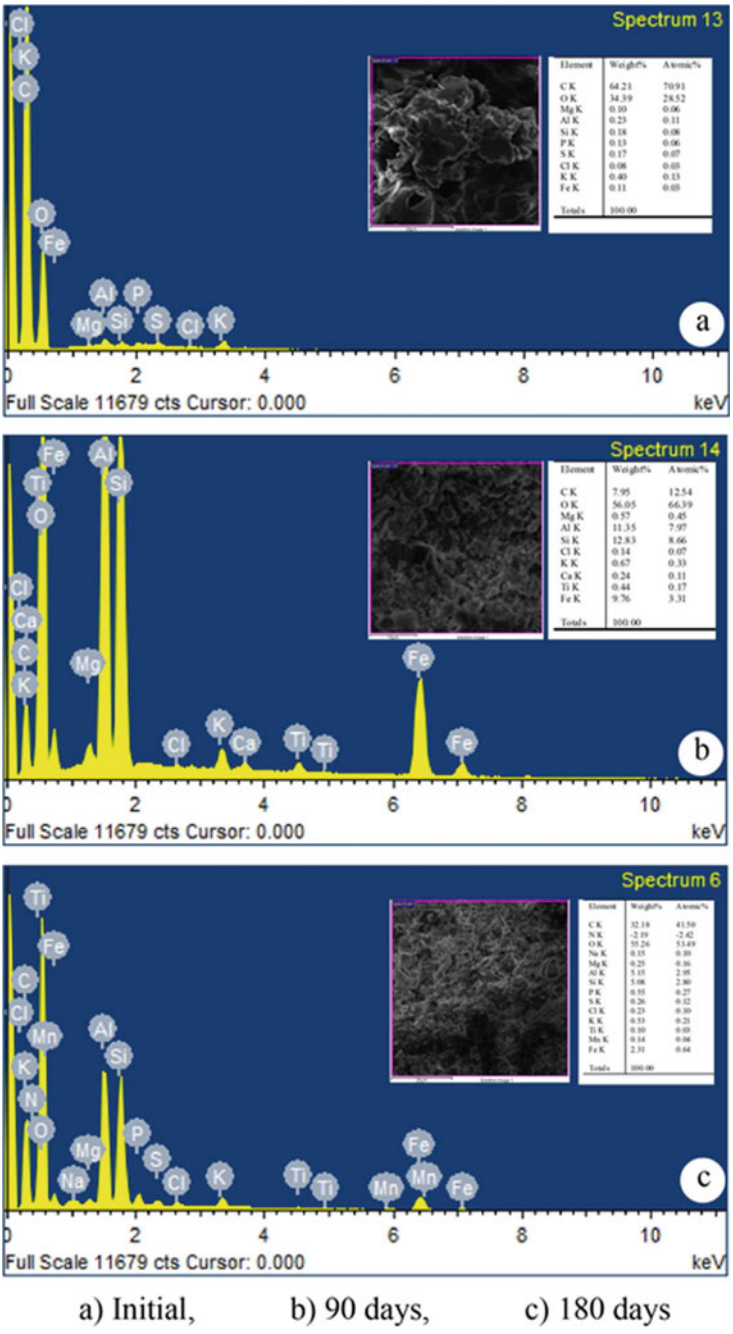

Fig. 7 SEM–EDX: elemental analysis of degradation of mixed samples of *Pinus radiata* Don

needle leaf, twig and bark of *Pinus radiata,* showed that the specific initial stage of elements are Si 0.18% and Fe 0.11%. In the 90th day of degradation the same elements shown higher levels of 12.83% Si and 9.79% Fe, whereas in the 180th day of degradation more elements are recorded, they are Al 5.5%, P 0.55%, K 0.53%, Ti 0.10%, Mn 0.14% and Fe 2.31%.

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