International Scholars Journals

African Journal of Wood Science and Forestry ISSN: 2375-0979 Vol. 4 (4), pp. 247-253, April, 2015. Available online at www.internationalscholarsjournals.org © International Scholars Journals

Full Length Research Paper

Contribution of a tropical fungus to premature failure of CCA treated *Eucalyptus grandis* poles in Kenya: Evidence of copper tolerance at Groundline attack

Sirmah Peter[1,3], Glassion Chelule[2], Hitimana Joseph[1] and Njue Naomi[1]

[1]School of Natural Resource and Environmental Management, University of Kabianga, P.O. BOX 2030-20200, Kericho, Kenya.
[2]Department of Forestry and Wood Science, University of Eldoret, P.O. BOX 1125-30100, Eldoret, Kenya.

Accepted 3 April, 2015

Studies were carried out to understand the contribution of fungi to premature failure of Copper Chrome Arsenate (CCA) treated *Eucalyptus grandis* poles used in Kenya for structural purposes. Wood chips from ground line contact in CCA treated *E. grandis* poles were cultured and re-cultured in laboratory under sterile conditions, 25°C and relative humidity of 75% for 10days. The fungus obtained was further cultured in 100, 500 and 1000ppm CCA in Potato Dextrose Agar (PDA) and growth inhibition evaluated. Fungal growth inhibition ranged from 38% to 82% at 100 to 1000ppm CCA respectively. Wood specimens treated with1000ppm CCA or not treated and exposed to the fungus in a standard laboratory soil bed gave weight losses of 8% and 28% respectively. It is concluded that the fungus was able to detoxify copper as evidenced by the formation of brown ring during initial stages of decay and growth. It is not only through copper detoxification and tolerance that the fungus is able to bring about premature wood failure. Soil pH, site factors and CCA leaching as reported in similar studies, work in a synergistic manner to accelerate the failure of CCA treated wood poles, explaining the huge losses experienced in Kenya.

Key words: CCA, tropical fungus, premature failure, *Eucalyptus grandis.*

INTRODUCTION

Copper Chrome Arsenate (CCA) is a waterborne wood preservative widely used in Kenya and Uganda to elongate the service life of wooden structures (Okwara, 2000; Venkatasamy, 2004; Ssemaganda, 2011). A large volume of CCA treated *Eucalyptus grandis* are used as electric transmission poles in Kenya, however fail prematurely at ground line resulting in great financial losses and dissemination of harmful chemical components into the environment (Chirenje *et al.,* 2004; Okwara, 2000). Previous studies attributed wood pole premature failure to site factors such as soil pH, climate, insects and fungi (Okwara, 2000).

Some wood decay fungi can still degrade wood treated with CCA (Kose and Kartal, 2010; Illman and Yang, 2004; Miha *et al*., 2004). Such behavior has been associated to the ability of fungi to synthesis, bind, absorb, tolerate and localize copper ions into their cell wall structures (Illman and Yang, 2004; Miha *et al.,* 2004; De Groot and Woodward 1999; Clausen *et al*., 2000; Green and Clausen, 2005; Köse and Kartal, 2010). Copper localization and tolerance by fungi has a significant practical impact on the long-term performance of treated wood products as well as the bio-processing of spent wood materials treated with copper-based preservatives (De Groot and Woodward, 1999). Indeed copper tolerance by brown-rot fungi is linked to oxalic acid production (a key fungal metabolite in the initial stages of wood decay) which presumably precipitates copper into an insoluble form of the oxalate, rendering the copper metabolite inert and appearing as a brown ring around the mycelia (Green III and Clausen, 2005; Clausen *et al*., 2000; Munir *et al., 2001*).

With the need to develop an environmentally friendly fungal bio-processing technology (Illman and Yang, 2010), recent research has focused on a search for fungi that can degrade CCA treated wood components (Illman

*Correspondingauthor.Email:sirmahkipkosgei110@hotmail.com

and Yang, 2004; Miha *et al.,* 2004). Identifying, understanding and isolating copper tolerant fungi will be at the base of the bioremediation of CCA-treated wood wastes (Illman and Yang, 2010). This study reports evidence of copper tolerance by a tropical fungus that is associated with premature failure of CCA treated *E. grandis* poles in Kenya.

MATERIALS AND METHODS

Sampling of Fungi

Ten CCA treated *Eucalyptus grandis* utility poles in service for the last 5-6 years and showing signs of fungal decay at ground line, were identified randomly in Kericho County, Kenya. Pole identification tags were read for treatment and installation data, while soil pH and site factors were as described by (Okwara, 2000). From each pole, wood chips (10mm ×2mm × 2mm) were carefully extracted from ground line contact using sterilized chisel, hammer and forceps. The chips were separately placed in tightly sealed sterile plastic containers and taken to University of Eldoret microbiology laboratories for culturing and further investigation.

Culturing of Fungi

Collected wood chips were surface sterilized to remove any contaminations by soaking in 10ml of 1% sodium hypochlorite (NaOCl) for 8 minutes, rinsed thoroughly 8 times using sterile distilled water until the associated smell of sodium hypochlorite disappeared. Under a sterile chamber three wood specimens were then introduced into 9cm petri-dishes containing 15 ml Potato Dextrose Agar (PDA) prepared as follows: 9.75gms of PDA (DIFCO Company) containing 50gms potatoes infusion, 5gms dextrose and 3.75gms agar was dissolved in 250ml distilled water in a conical flask . The solution medium was autoclaved (1hr, temperature of 121℃ and 15psi pressure). The experiment was replicated three times. All petri -dishes were incubated in a sterile chamber at 24°C- 25°C and relative humility (RH) of 65%- 70% for 10 days. Mycelia from petri- dishes showing fungal growth were isolated using a needle previously sterilized through heating over a spirit lamp. The mycelium isolate was then inoculated on the centre of a petri- dish with freshly prepared PDA medium. This aimed at isolating a CCA tolerant fungus, re-cultured to give clearly propagated mycelium and which was used in the next stage of this study.

Fungal Growth in a CCA inoculated PDA medium

CCA (Osmose C) wood preservative was prepared to (0, 100, 500 and 1000ppm) following the standard procedure as described by Mburu *et al.,* 2007. Under sterile conditions, 2ml of each CCA concentration was mixed with 15ml PDA medium in separate petri-dishes, labeled and allowed to solidify. A 1mm cube from the edge of fully grown fungi (isolated as previously described) was placed at the centre of a 9cm petri- dish, sealed and incubated at 24 °C - 25 ℃ and RH of 65%-70% for 10 days. Fungal growth was evaluated once every 2 days by measuring two vertical diameters of the colony and expressed as follows:

$$\%\ growth\ inhibition = 100\ (1 - \frac{di}{do})$$

where d_0 is the diameter of the control culture and d_1 the diameter of the culture in the presence of CCA. All experimental units were replicated thrice.

Wood Resistance Against Tropical Fungus in a Soil-Bed Tests

Wood Specimen Preparation

This study was based on a modified AWPA: E23-07 (American Wood Protection Association, 2008) test standard. One hundred and twenty eight (128) clear sapwood *Pinus patula* (a perishable wood) specimens, 50mm × 10mm × 5mm, (l × r × t) were prepared. To understand fungus tolerance on CCA wood preservative, thirty two *P. patula* wood specimens were treated using none (0), 100, 500, and 1000 ppm of CCA separately. All the wood specimens were conditioned at 60 ˚ C in an oven before and after CCA treatment to constant mass (w_2 and w_o) respectively and labeled for identification purposes.

Soil Bed Preparation and Exposure of Wood Specimens

Plastic containers 250mm × 150mm× 170mm (l, w, h) were filled with unsterile soil layers collected from around the *E. grandis* utility poles in Kericho from bottom to top as described by (Kibet, 2013) and briefly as follows: 20mm of gravel, 20mm of sand and 130mm of the collected soil.

The *P.patula* wood specimens prepared as described earlier were then planted randomly 20mm apart so that approximately 1/3lenght of each specimen protruded above the soil bed level. The soil beds were kept under room temperature (22℃-28℃) and controlled soil moisture of about 70% that is reported to optimize fungal wood decay (Kibet, 2013). Soil beds were weighed weekly and sufficient water added to bring the weight of the container back to the original weight that gave the required moisture content.

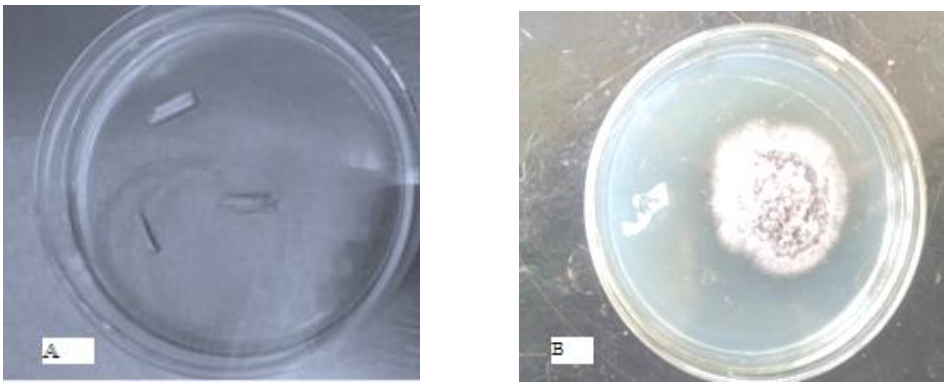

Figure 1 . *E. grandis* wood chips (A) before and (B) after culturing.

Fungal Wood Decay Resistance Assessment

Fungal wood decay was assessed by evaluating the mass losses once every four weeks for 32 weeks. During each inspection a replicate set of 4 specimens from each CCA concentration level (0, 100, 500, 1000 ppm) were uprooted from the soil-bed cleaned from any mycelium and soil particles which adhered to the surface . The specimens were then oven -dried at 102±3℃ for 48 hours and the mass loss evaluated as a percentage of the original mass before exposure using the formula below:

$$Mass\ loss, \% = \frac{W_0 - W_1}{W_0} \times 100$$

where, w_1 and w_0 is the weight of test specimen after

and before exposure to fungal decay respectively.

RESULTS AND DISCUSSIONS

Cultured Tropical Fungi

Figure 1 above shows fungal growth on CCA treated *E. grandis* wood chips before and after culturing in PDA medium and incubating for 10 days at RH of 65- 75%.

The ability of the fungus to proliferate in *E. grandis* chips from utility poles previously treated with CCA (vacuum 650 mmHg for 30 minutes and pressure of 10 bars for 3 hours) to retention of 20 kg/m^3 indicate its ability to tolerate copper ions, a known fungicide. Formation of a brown ring around the fungal mycelia in figure 1(B) indicates production of oxalic acid, a key fungal metabolite in the initial stages of wood decay (Munir *et al.,* 2001)

Fungal Growth in CCA Inoculated PDA medium

Figure 2 below shows fungal mycelium growth in PDA media inoculated with different CCA concentrations and incubated for 2 to 10 days at RH of 65- 75% in a sterile chamber.

Significant mycelium growth was observed in PDA media inoculated to different CCA concentrations level. At the higher concentration of 1000ppm CCA, least growth in perpendicular diameter of fungus mycelia was observed. Indeed mycelium growth increased with decreasing CCA concentration. Full growth of the fungus mycelia and hyphae were observed in petri dishes without CCA inoculation after 10 days. As the fungus continued to grow and thrive at different CCA concentrations, red coloration at the periphery of the mycelium was observed, indicating production of oxalic acid described in literature as a positive indicator of copper tolerance (Green III and Clausen, 2005*;* Clausen *et al*., 2000; Munir *et al. 2001*). Indeed, metal-tolerant macrofungi are capable of causing the rot of CCA preservative-treated wood. In their study, Illman and Yang (2010), observed that *Merulipona incrassate* and *Antrodia radiculosa* fungi were able to degrade CCA treated wood to more than 20% of the original dry weight of the wood. Such microfungi could develop on CCA treated wood, destroy toxic matters and neutralize their action, hence suitable for biodegradation of wood waste (Illman and Yang, 2010; Köse and Kartal, 2010; Miha *et al*., 2004).

Figure 3 below shows the ability of CCA to inhibit the growth of the tropical fungus. In all test days, higher and least growth inhibition was observed at 1000ppm and 100ppm CCA respectively. In spite of growth inhibition, the tropical fungus was able to detoxify the copper ions and grow.

This suggests that the tropical fungus under investigation had greater potential of proliferation through detoxification of copper ions. The tolerance of the fungus in the study showed varying inhibitory characteristics depending on the concentration of CCA. The observed initial inhibition and subsequent fungal proliferation progressively to the tenth day can be associated with detoxification of the CCA agar medium by fungal enzymes (Neya *et al., 2004*; Gerardin *et al.,* 2004). These characteristic phenomenon of fungi to resist biocides

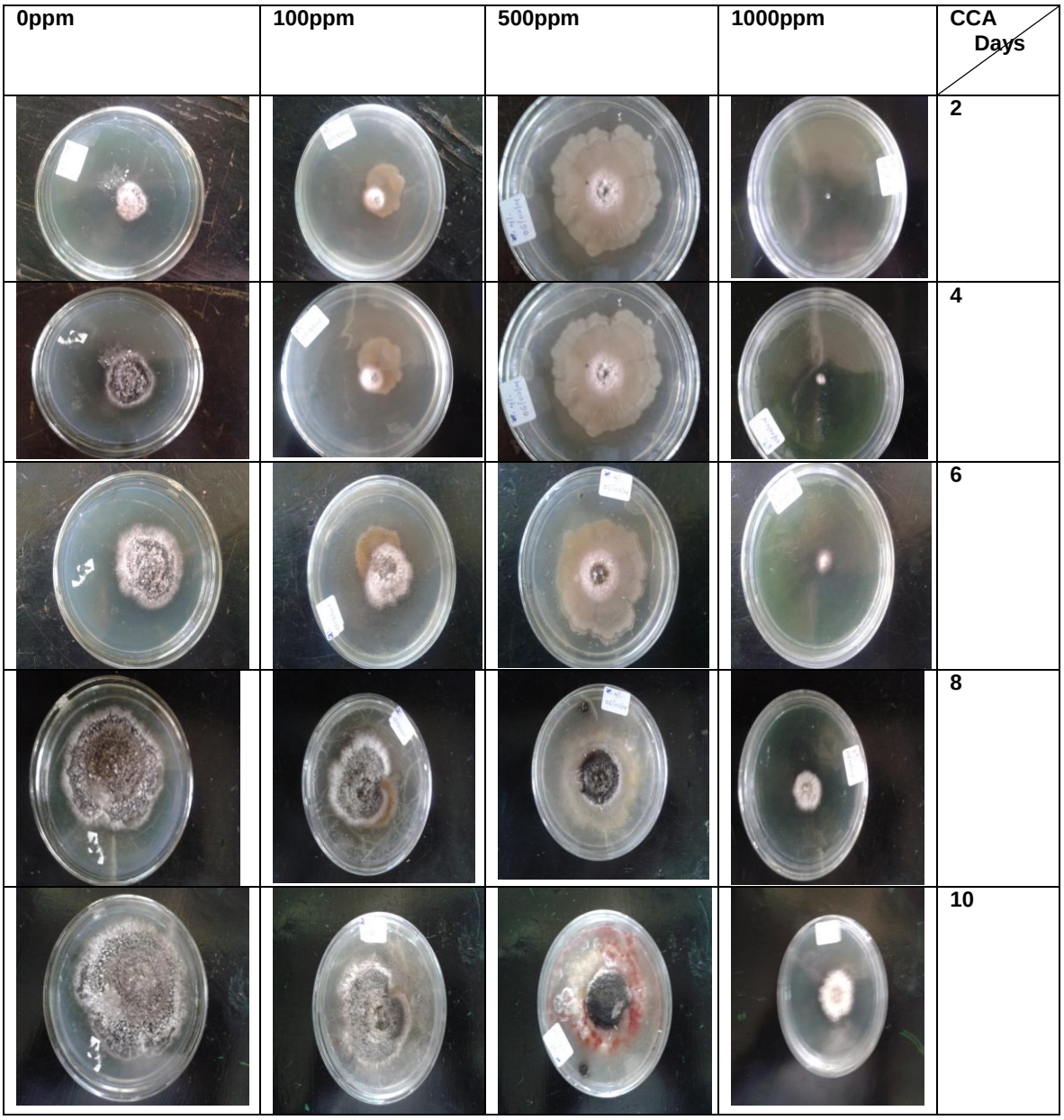

Figure 2. Fungal mycelium growth in PDA media inoculated with CCA at different days.

have been attributed to diverse mechanisms involving the trapping of the metal by cell-wall components, altered uptake of copper, extracellular chelating or precipitation by secreted metabolites, to intracellular complexing by metallothioneins and phytochelatins, although only the metallothioneins chelation mechanism has been approached with molecular detail (Illman and Yang, 2004; Miha *et al.,* 2004; De Groot and Woodward 1999; Clausen *et al*., 2000; Green and Clausen, 2005; Köse and Kartal, 2010). Indeed Köse and Kartal (2010) suggest that the high copper tolerance of some wood rot fungi are due to their habit of producing oxalic acid which, it was supposed precipitates the copper into the insoluble form of the oxalate thus rendering the copper metabolite inert.

Wood Mass Loss from Soil Bed Test

Figure 4 below is the appearance of *P. patula* wood blocks before and after exposure to the tropical fungus in a soil bed test.

The wood blocks appeared discolored and soft indicating attack by the fungus and is consistent with re-

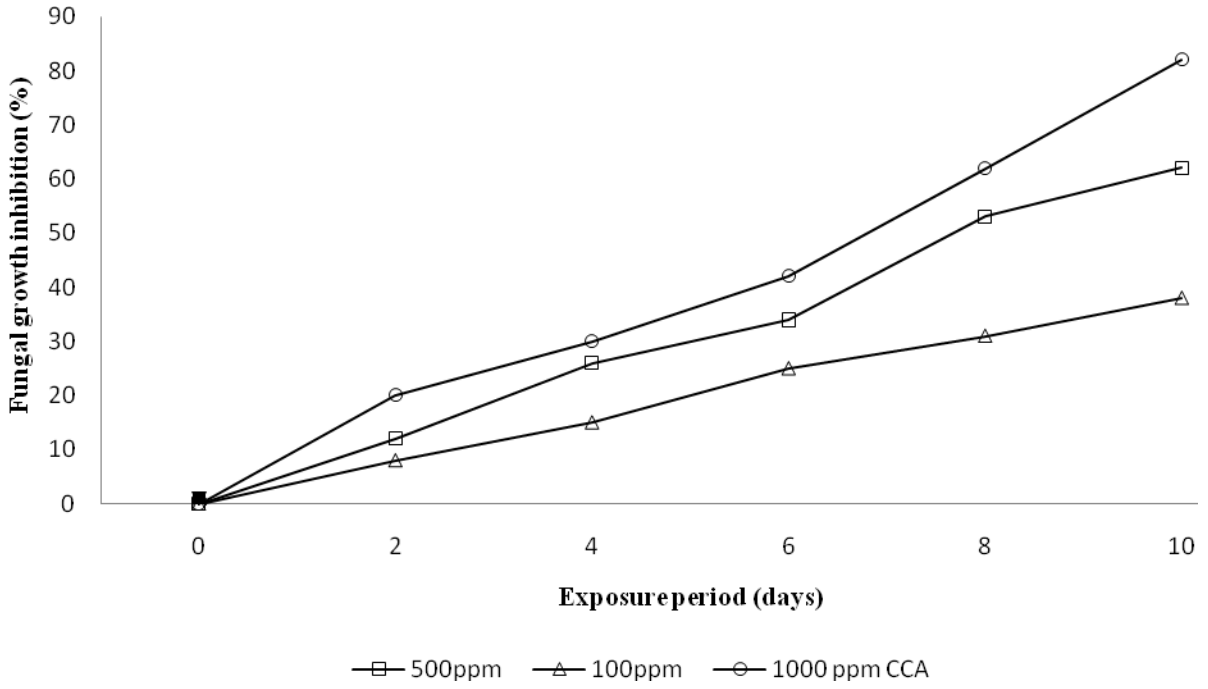

Figure 3. Growth inhibition at different CCA concentrations

Figure 4. *P. patula* wood blocks A) before and B) after exposure to the tropical fungus in a soil bed test.

ports by earlier workers (Kibet, 2013; Mburu, 2007). The wood mass losses due to fungal degradation in the soil bed are reported in Figure 5 below:

The first 4 weeks of exposure showed little variability in decay with percentage mass loss on all test specimens of about 2.04%. This unclear decay pattern during initial period of wood exposure in soil bed test has been reported for other species (Acker *et al.* 2003; Kibet, 2013; Lebow *et al.,* 2004; Lebow *et al.,* 2000). In the next eight weeks, mass loss on all wood blocks increased tremendously, followed by an immense mass loss on untreated blocks in the next 16 weeks. In all the test blocks, mass losses due to fungal decay increased with exposure period with a significant difference amongst the treatments ($p<0.05$). This is in agreement with observation that mass loss in softwoods is generally low in soil bed test (Acker *et al.,* 2003; Machek *et al.,* 2001; Kibet, 2013). In our current experiment, treatment of wood

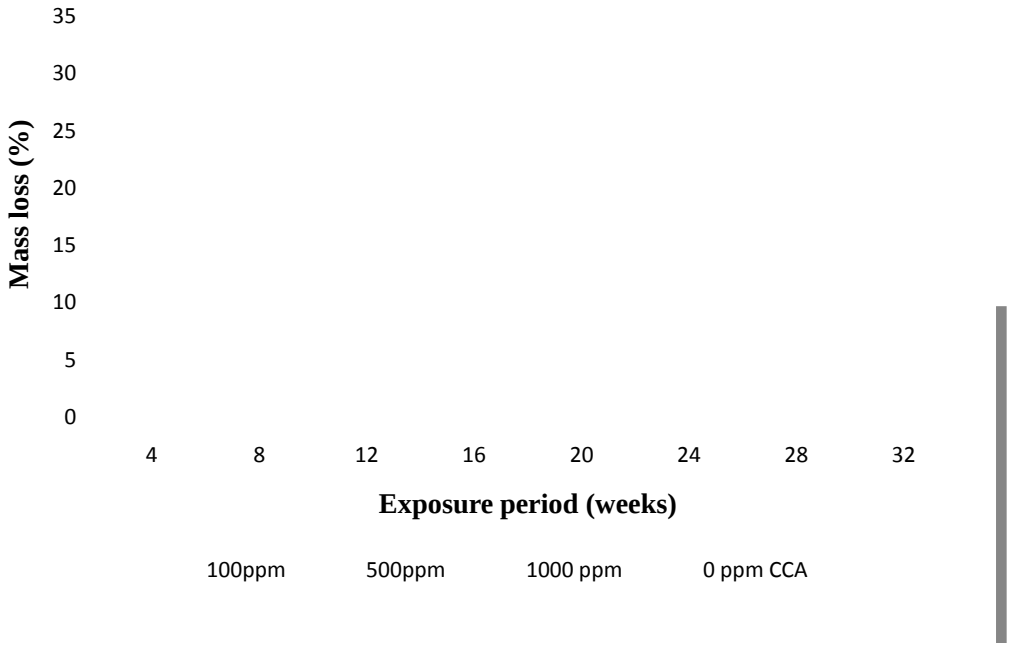

Figure 5. Percentage mass loss of wood specimens in the fungal soil-bed.

blocks with CCA lead to significantly lower mass losses ($p<0.05$), suggesting fungal tolerance and detoxification periods. It is therefore clear that the tropical fungus was able to degrade wood blocks even in the presence of the CCA wood preservative. Generally, the mass loss increased with decreasing CCA concentration implying that the tropical fungus was able to detoxify this preservative at all levels.

CONCLUSION

Unidentified tropical fungus associated with premature ground line failure of CCA treated *E. grandis* poles in Kenya has been successfully cultured. The fungus isolated from *E. grandis* wood poles treated initially to CCA retention of 24 kg/m^3, was able to detoxify copper ions and thrive at 1000ppm CCA in laboratory and soil bed tests. Once copper is detoxified, the wood is open to attack by other wood degraders. Put together, these results suggest that the reported premature failure of *E. grandis* utility poles in Kenya is partly contributed by CCA tolerant fungi, hence a need to consider other more novel wood preservatives. These findings are limited by CCA leaching, site factors and soil pH as reported in other literature that may also contribute to the premature failure of poles in service.

REFERENCES

Acker J, Stevens M, Carey J, Siera-Alvariez R, Militz H, Bayon I, Kleist G, Peek R (2003). Biological durability of wood in relation to end-use. Holz als Roh-Und Werkstoff. 61:33-45.

AWPA: E23-07 (2008). Accelerated method of evaluating wood preservatives in soil contact 2008.

Chirenje T, Ma LQ, Szulczewski M, Hendry K, Clark C (2004). Retention of As, Cu, and Cr leached from CCA-treated wood products in select Florida soils. Proceedings environmental impacts of preservative treated wood, Feb 8-11, Orlando Florida, USA p. 45-56.

Clausen CA, Green III F, Woodward BM, Evans JW, DeGroot RC (2000). Correlation between oxalic acid production and copper tolerance in *Wolfiporia cocos*. International biodeterioaration and biodegradation. 46: 69-76.

DeGroot RC, Woodward BM (1999). Using Copper tolerant fungi to biodegrade wood treated with copper based preservatives. International biodeterioaration and biodegradation. 44: 17-27.

Gerardin P, Neya B, Dumarcay S, Petrissans M, Mohamed S, Huber F (2004). Contribution of gums to natural durability of *Prosopis africana* heartwood. Holzforschung. 68:39-44.

Green III F, Clausen CA (2005). Copper tolerance of brown-rot fungi: Oxalic acid production in Southern pine treated with arsenic-free preservatives. International biodeterioration and biodegradation. 56: 75-79.

Hoang C, Nguyen T, Stanley D, Persily A, Corsi RL (2010). Effect of Ozonation on the fungal resistance of bamboo and oak flooring materials. Building and environment. 81: 226-233.

Illman B, Yang V (2010). Mycoremediation of waste wood treated with CCA and ACQ. Proceedings Seventh international conference on remediation of chlorinated

and recalcitrant compounds preliminary program, March 24-27, Monterey, CA. Battelle CA, USA.
Illman B, Yang V (2004). Bioremediation and degradation of CCA treated wood waste: Proceedings environmental impacts of preservative treated wood, Feb 8-11, Orlando Florida, USA p. 259.
Kibet S, Sirmah P, Mburu F, Muisu F (2013). Wood dimensional stability and extractives as reasons for termite and fungal resistance of the lesser known *Albizia malacophylla* Kenyan wood species. J. of the Indian Acad. of Wood Sci., 10(1): 48-54.
Köse C, Kartal SN (2010). Tolerance of brown-rot and dry-rot fungi to CCA and ACQ wood preservatives. Turk. J. Agric. For., 34:181-190.
Lebow ST, Copper P, Lebow P (2004). Variability in evaluating environmental impacts of treated wood. Proceedings environmental impacts of preservative treated wood, Feb 8-11, Orlando Florida, USA pp. 3-16.
Lebow ST, Nilson T, Morell JF (2000). Performance of copper based wood preservatives in soil bed exposures. Material and Organism. 33, 235-244.
Machek L, Militz H, Sierra-Alvarez R (2001). The use of an acoustic technique to assess wood decay in laboratory soil-bed tests. Wood Sci. Technol., 34: 467-472.
Mburu F, Durmarcay S, Huber F, Petrissans M, Gerardin P (2007). Evaluation of thermally modified Grevillea robusta heartwood as an alternative to shortage of wood resource in Kenya: Characterization of physicochemical properties and improvement of bioresistance. Bioresource technology. 98: 3478-3486.
Miha H, Pholeven F, Armatey OS (2004). Bioremediation of waste copper/chromium treated wood using decay fungi. Proceedings environmental impacts of preservative treated wood, Feb 8-11, Orlando Florida, USA pp. 245-258.
Munir E, Yoon JJ, Tokimatu T, Hatton T, Shimada M (2001). A physiological role for oxalic biosynthesis in the wood rotting basidomycete *Fomitopsis palustris*. PNAS. 98: 1126- 11130.
Neya B, Hakkou M, Petrissans M, Gerardin P (2004). On the durability of *Burkea africana* heartwood : Evidence of biocidal and hydrophobic properties responsible for durability. Ann. For., 61: 277-282.
Okwara DN (2000). Effects of site factors on the performance of Copper Chrome Arsenate (CCA) treated *Eucalyptus saligna* telegraph poles. Master's thesis, Moi University, p. 92.
Ssemaganda IE, Mugabi P, Tumwebaze SB (2011). Effectiveness of selected preservatives in protecting Ugandan growing *Eucalyptus grandis* wood against termite attack. Maderas, Ciencia y technologia. 13(2): 135-142.
Venkatasamy R (2004). Unregulated use of toxic wood preservatives chemicals in Kenya: Health and environmental issues. Proceedings environmental impacts of preservative treated wood, Feb 8-11, Orlando Florida, USA p. 375.