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Effect of site, species and tree size on the quantitative variation of lipophilic extractives in *Eucalyptus* woods used for pulping in South Africa

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ABSTRACT

Lipophilic wood extractives have serious negative impacts on both pulping process and quality of produced pulp. This study aimed at identifying suitable wood materials for pulping with respect to their lipophilic extractives contents. The effect of site, species and tree sizes on the amount of lipophilic extractives was evaluated. The lipophilic extractives from selected *Eucalyptus* species used for pulping in South Africa were quantified using gas chromatography–mass spectrometry. It was revealed by the use of analysis of covariance (ANCOVA) that the quantitative variation of lipophilic extractives in *Eucalyptus* woods is significantly affected by respective sites and tree species. Principal component analysis (PCA) revealed the correlation of the amount of lipophilic extractives in wood materials with tree species/clones and site soil composition. Thus, high amounts of lipophilic extractives were found in *Eucalyptus* trees grown at sites with a high composition of clay soil and organic matter. Whereas, *Eucalyptus dunnii* was found to contain a higher amount of lipophilic extractives than *Eucalyptus grandis* in all the sampled sites, implying an increased risk of pitch formation during the pulping process.

1. Introduction

Eucalyptus trees are among the most common hard wood sources of fibres for chemical cellulose and paper production in South Africa. The attractive features of *Eucalyptus* trees include their ability to grow in a wide range of environmental conditions, rapid growth and the excellent properties of the pulp produced (Prinsen et al., 2012; Santos et al., 2013; Silvério et al., 2007a). The quality of the pulp depends on the raw materials and pulping process used, thus the choice of tree species and/or clones for pulping is of great importance. The variations in pulp quality are due to the effects caused by the variations in amount and chemical composition of the raw materials. Trees undergo variations in their chemical composition during different major stages; the properties of the living tree vary and affect pulp properties and pulping operations (Silvério et al., 2008). The variations in the chemical composition of the living tree are caused by a number of factors, including the quality of the site, season of harvesting, geographical location of the plant, climate, age of the tree and species/clone (Bikovens et al., 2013; Doussot et al., 2002; Silvério et al., 2007a).

Apart from cellulose, tree wood materials contain other structural and non-structural components such as hemicelluloses, lignin and wood extractives (Chen et al., 2010). Among these non-cellulosic components, wood extractives, particularly lipophilic extractives, are very resistant to the pulping chemicals and normally survive the process (Sitholé et al., 2010) causing a very serious negative impact on the pulping process and quality of produced pulp. Generally, high content of lipophilic extractives in wood materials cause high consumption of bleaching chemicals, pitch deposition in the pulping machines leading to the suspension of production for maintenance and servicing of pulping equipments. The residuals of lipophilic extractives in final produced pulp cause black spots. They are also responsible for the effects on the physicochemical properties of fibres such as decreased surface energy, as extractives enriched on the fibre surfaces tend to decrease the fibre-fibre bonding ability (Asikainen et al., 2010; Gutiérrez et al.,

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2001; Silvério et al., 2007a, 2008). They also affect the soil pH when pulp mill effluents are released into the environment (Gutiérrez et al., 2001; Suominen et al., 2003), thus with the potential to affect aquatic organisms due to their toxicities (Orrego et al., 2010). Lipophilic extractives include mainly fatty acids, sterols and their esters; as well as fatty alcohols, triglycerides, hydrocarbons, steroid hydrocarbons and ketones (Challinor, 1996; Freire et al., 2005; Horvath, 2006; Poke et al., 2004; Sitholé et al., 2010). *Eucalyptus grandis*, *Eucalyptus dunnii* species and *E. grandis* × *E. urophylla* clone (GU) are among the species and clones used for pulping in South Africa. For instance, *E. grandis* is the most extensively cultivated hardwood species in South Africa (Hajari, 2004; Komakech et al., 2007; Komakech, 2008). *Eucalyptus grandis* and *E. dunnii* have a similar growth rate (referred to as fast-growing *Eucalyptus* species) on the same climatic conditions (Smith et al., 2006). However, to the best of our knowledge, the similarities and differences of these species and clones used for pulping in terms of their lipophilic extractive contents with respect to sites and tree sizes have not been explored. Moreover, not many studies that have investigated the effect of site quality, species and tree sizes on the lipophilic extractive contents and variations in *Eucalyptus* species and clones grown in South Africa have been published thus far. In one of the previous publications, it was reported that the size of a tree and its height above the ground is significant to the tree as it determines the total amount of light that the tree is able to harness for photosynthesis (Arzai and Aliyu, 2010) which will to a large extent affect the plant physiology and consequently affect plant chemical composition. Pulping process can be smoothly done if the tree-wood materials contain fewer amounts of lipophilic extractives. Thus, assessment of the effect of the site, species/clones and tree sizes on the amount of lipophilic extractives in the wood materials will assist in identifying the sites, species and tree sizes suitable for pulping with respect to the content of lipophilic extractives. Different solvents have been used in the extraction of lipophilic extractives from solid wood samples of which, the choice is based on the extraction ability. For example, acetone has been reported to have high extraction ability compared to other organic solvents (Freire et al., 2005; Sun and Tomkinson, 2003; Thurbide and Hughes, 2000). Although, its extracts contain other component classes apart from lipophilic extractives, such as lignans and low molecular weight carbohydrate, its lipophilic extractive components can be isolated by dissolving the total acetone extracts into solvents, such as diethyl ether (Lužáková and Vrška, 2005) dichloromethane (Silvério et al., 2007a,b), chloroform (Gutiérrez et al., 2008; Marques et al., 2010).

This study therefore, investigated the contribution of tree sizes, location and also species/clones in the types and amount of lipophilic extractives with the aim of identifying factors that can reduce the presence of extractives in the pulp. Principal component analysis (PCA) was used to reveal the correlation of the amount of lipophilic extractives in wood materials with tree species/clones and site soil composition.

2. Materials and methods

2.1. Wood samples

Eucalyptus fresh tree samples were collected from four different sites in KwaZulu-Natal Province in South Africa, namely Zululand (for GU clone), Umvoti, Ixopo (Breamer and Sutton) and Richmond sites (for *E. grandis* and *E. dunnii*). Sampling of the trees involved identifying the blocks within the respective site and then the trees to be felled. Trees were then; sampled with respect to their sizes (i.e. small, medium and large) based on their diameter at breast height (DBH) values. The identified plants were felled and discs of 3 cm thick were cut from breast height (1.3 m above the ground). Fresh tree discs collected were debarked, chipped into small sized pieces and kept frozen until such time as they were analysed. The height of the felled trees was directly measured using a linear tape measure, whereas their diameter at breast height (DBH) was determined by measuring the circumference of the tree trunk at a height of 1.3 m above the ground and then calculating the BDH value.

2.2. Soil samples

Soil samples were collected around each tree that was sampled in order to determine soil-composition measurements which could assist in accounting for the variation in site qualities. The soil parameters measured included pH, organic matter and soil-grain composition, of which their correlations to the lipophilic extractives with respect to wood samples were evaluated using chemometric methods. Samples were collected at the soil surface and at a depth of between 1 m and 2 m, depending on the nature of the site.

2.3. Sample extraction

The air dried wood samples which were previously treated with distilled water (i.e. Fresh wood chips were ground into small particles and soaked in distilled water and shaken on the auto-shaker to remove water-soluble tree components such as, protein, colouring matter, sugars and volatile compounds) to remove all water soluble compounds, were extracted by Soxhlet using acetone. About 2 g of sample (in duplicate) was placed in a Soxhlet thimble and extracted for 6 h. Solvent was then evaporated to dryness using a rotary evaporator, and the extract weighed and re-dissolved in 2 mL chloroform to isolate lipophilic extractives from the total acetone extracts. Chloroform solution was filtered using PTFE disc filters of 0.2 μm, and the solvent evaporated using nitrogen gas and re-dissolved into 0.5 mL acetone for derivatization before gas chromatography mass spectrometry analysis. Moisture content of the wood samples was determined by drying the same weight of the samples in an oven at 105 °C to constant weight.

2.4. Derivatization

For the identification of wood extractives by GC–MS, a derivatization step was necessary and done using 3 M methanolic HCl which was added to the sample at a ratio of 1:2 (methanolic HCl: sample), respectively. Undecanoic acid and cholesterol mixture internal standard was added before derivatization. The mixture was then heated at 60 °C for 1 h in a water bath, cooled at room temperature and followed by solvent evaporation to dryness using nitrogen gas, then re-dissolved in 0.3 mL of HPLC grade methanol, and then filtered using PTFE disc filters of 0.2 μm porosity to eliminate any un-dissolved particles, before the GC–MS analysis.

2.5. GC–MS conditions

A Shimadzu (GCMS-QP2010, Kyoto, Japan) GC-MS was used for the separation and detection of the lipophilic extractives. A ZB-1MS column [30 m × 0.25 mm i.d., 0.25 μm film thickness] was used for separation, while UHP helium served as a carrier gas at a flow rate of 1.0 mL/min. Injection volume was 1 μL, while the injector temperature was set at 250 °C, and the oven temperature programme was as follows: initial temperature set at 60 °C and held for 1 min, then ramped up to 290 °C at a ramp-up rate of 15 °C/min and held for 10 min at a linear velocity of 36.4 cm/s. The interface temperature was set at 250 °C and the ion source temperature at 240 °C. Compounds were identified based on the comparison of the mass spectra with those recorded in the NIST libraries (NIST 2005), and