Lignin ammoxidation: Synthesis of nitrogen releasing soil conditioning products from waste pulp liquor and their pot trial evaluation

Luvuyo Tyhoda (✉ ltyhoda@sun.ac.za)

Stellenbosch University <https://orcid.org/0000-0003-4514-2283>

Qiniso Ngiba

Stellenbosch University

Johann F. Görgens

Stellenbosch University <https://orcid.org/0000-0002-9961-754X>

Research Article

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Abstract

Purpose This study investigated the ammoxidation of three industrial lignins of which two were respectively precipitated from kraft and soda liquors, and the third one, a filtered sodium lignosulfonate for the synthesis of soil conditioning materials.

Method The industrial lignins were characterized for structural properties and ammoxidized in a 1 L Parr reactor at 80 °C, 10 barg, for 4 hours, with 7 wt. % ammonia and 10 wt. % lignin in the reaction mixture. A plant trial assessment of the products was conducted over four weeks.

Results N-lignins with C/N ratios of 12.30, 15.81, and 14.64 were obtained from kraft, soda and sodium lignosulfonate against a standard requirement of a C/N < 20. However, soda lignin and sodium lignosulfonate could not meet the criteria for N-lignins (C/N < 20) under standard reaction conditions, requiring an additional pre-oxidation with 5% hydrogen peroxide prior to ammoxidation. In the plant trial, N-modified kraft (3.50 t/ha) and soda lignin (3.21 t/ha) recorded crop yields that were 71% and 57% higher than the control (2.04 t/ha), respectively. The sodium lignosulphonate, while it met the requirements for use as a soil fertilizing material in terms of nitrogen content, resulted in complete crop failure. Further characterization showed that due to its high pH (8.81 pH) and a high salt index (63.62%) due to the pulping technique used in its isolation, was unsuitable as a raw material for soil fertilizing materials.

Conclusion Kraft and soda lignins could be successfully ammoxidized to synthesize N-lignins that are suitable for use as soil conditioning materials.

Statement Of Novelty

The goal of chemical pulping is to remove portions of the lignin and hemicelluloses from biomass to produce a cellulose-rich pulp. Spent liquors, which contains modified lignin fragments, degraded sugars, and residual pulping chemicals are also produced as by-products of the process. Lignin is a precursor of soil humus, making spent pulping liquor an attractive starting material for the synthesis of soil conditioning/rehabilitation products through a process called oxidative ammonolysis or ammoxidation. Ammoxidation incorporates nitrogen into lignin, making it easier to degrade in the soil. The present study investigated the ammoxidation of lignins isolated from alkaline pulping liquors (kraft and soda) as well as a neutral sulphite semi-chemical pulping lignosulphonate, which have not been investigated for this process previously.

1. Introduction

The decline in soil productivity demands intensive agricultural management practices to maintain or increase crop yields to feed the growing global population [1, 2]. Intensive agricultural practices such as tillage, low harvest residue retention, and the over-application of mineral nitrogen fertilizers lead to an unsustainable mineralization of soil organic matter (SOM) and high erosion rates [1].

SOM facilitates the formation of soil aggregates, improves cationic exchange capacity, and supports the soil microbial community. Its rapid mineralization disturbs the soil structure, depletes soil nutrients, makes the soil prone to erosion, and limits biological activity, leading to a decline in soil productivity [5, 6]. Increasing the nitrogen application rates to increase crop production may accelerate SOM mineralization, further lowering soil fertility. The natural formation of humus – the stable form of SOM is very slow, especially in areas suffering from severe degradation [3, 4]. It is thus imperative to synthesize organic amendments to improve SOM in agricultural land and to rehabilitate degraded soils, while also ensuring that these products have slow-nitrogen-release properties [9].

Soil organic amendments have the potential to affect soil nitrogen dynamics. The application of organic amendments that have a carbon-to-nitrogen (C/N) ratio that is higher than 30 can result in a net immobilization of nitrogen in the soil, causing nitrogen deficiencies for crops [7 – 11]. Conversely, organic amendments that have a C/N ratio less than 25 can cause a net mineralization of nitrogen in the soil - making nitrogen available for plant uptake and increasing SOM content. Only organic amendments with a C/N less than 20 are considered acceptable for soil application [9].

Nitrogen-releasing, soil-conditioning materials have been synthesized from organic substrates through a process called ammoxidation. The products of this process are referred to as N-lignins (synthesized from lignin) or NovihumTM (synthesized from lignite). In this process, the raw material is treated with oxygen and ammonia, binding nitrogen into it in different functional groups. Nitrogen is made plant-available through the degradation of the molecular structure of these products by soil microorganisms, and through the chemical degradation of functional groups (e.g., hydrolysis) [10]. These functional groups mineralize at different rates in the soil, resulting in a nitrogen slow-release characteristic [11, 15, 16]. Unlike lignite, lignin is a renewable raw material and a precursor of soil humic substances [17, 18]. Furthermore, the application of lignin in the soil has also been reported to increase erosion resistance and inhibit nitrification [10, 19].

Ammoxidation is analogous to natural humus formation. Both these processes involve oxidation, demethylation, demethoxylation, cleavage of aromatic structures, and the formation of muconic acid derivatives [9]. Various technical lignins have been used as raw materials for ammoxidation, yielding products with 3–5% nitrogen, and C/N ratios less than 20 [16, 17, 21]. The properties of the raw lignin affect its suitability to ammoxidation. Lignins with functional groups that are reactive to oxidation (hydroxyl groups and methoxyl groups) incorporate higher nitrogen contents during ammoxidation. The ammoxidation of reducing sugars leads to the formation of growth-inhibiting substances. High reducing sugar content in the raw lignins is thus a limitation to their utilization for N-lignin synthesis [9].

The aim of this study was to assess the suitability of the main industrial lignins available in the South African pulping industry, obtained from kraft, soda and NSSC pulping process for use as raw materials in the synthesis of N-lignins through a typical ammoxidation method. While previous studies have shown that acid sulphite lignins are not suitable starting materials for the synthesis of N-lignins, lignosulfonates from NSSC pulping have not been studied previously [9, 12]. The characteristics of the raw lignins

(functional group content, ash content, and elemental composition) were used to predict the performance of these raw materials during ammoxidation. The synthesized N-lignins were assessed for nutrient uptake and toxicity in a plant trial using white mustard (*Sinapis alba*) over a four-week period.

2. Materials And Methods

2.1 Materials

The following spent liquor samples were collected from South African pulp and paper mills:

- Kraft pulping liquor obtained from Mondi, Richards Bay (hardwood biomass)
- Soda pulping liquor from Sappi, Stanger (sugarcane bagasse)
- Sodium lignosulfonate liquor obtained from Mpact, Piet Retief (Neutral sulfite semi-chemical (NSSC) pulping of a mixture of hardwood and softwood biomass)

2.2 Methods

2.2.1 Lignin precipitation and purification

Kraft lignin (KRT) and soda lignin (SOD) were acid precipitated from their liquors at pH 4 using concentrated sulphuric acid, according to a method reported by Naron *et al.* (2017) [17]. The liquors were kept at pH 4 for 24 hours and centrifuged at 7000 rpm for 10 mins to recover the lignins. The lignins were washed with reverse osmosis water and recovered by centrifugation. This step was repeated twice, after which the lignins were air-dried and milled using a Retsch ZM 200 ring and puck mill (0.5 mm aperture size, at 6000 rpm). These materials were further purified by soaking, over a 24-hour period, in a 0.5 M sulphuric acid solution in a ratio of 20 L of sulphuric acid solution per 100 g lignin. The suspension was subsequently vacuum filtered using type 1 Whatman filter paper, washed with reverse osmosis water, and air-dried. Sodium lignosulfonate (SLS) was filtered to using type 1 Whatman filter paper to remove debris and used without further purification.

2.2.2 Lignin characterization

The ash content of the raw lignins and the synthesized N-lignins was determined according to NREL/TP-510-42622. The elemental compositions of the raw and ammoxidized lignins were determined according to ASTM D4239 (sulfur) and ASTM D5373 (carbon, hydrogen, and nitrogen) methods.

Fourier Transform Infrared Spectroscopy (FTIR) was used to investigate structural features that each raw material possessed, using a Bruker Alpha II ATR-IR instrument. A background measurement was performed before each experimental spectrum was recorded. Data was recorded for each sample in the range 4000 - 400 cm^{-1} using a resolution of 4 cm^{-1} . Absorption peaks were identified, and the corresponding functional groups were identified based on literature [23, 26, 28].

The reducing sugar content of the starting materials was determined using the phenol sulphuric assay (AOAC method 988.12) [21]. Sample solutions were mixed with 1 ml of 5% phenol solution and 5 ml of 98% sulphuric acid and kept at 30 °C for 20 minutes. The absorbance of each sample mixture was then read from a UV-Vis spectrophotometer at 480 nm. The sugar content was determined using a calibration curve constructed using known xylose concentrations.

The weight average molecular weight (M_w) of the raw lignins was determined through size exclusion chromatography, using an Agilent 1260 infinity Quaternary LC unit. The solvent was a mixture of dimethyl sulfoxide (DMSO) and water (9:1, v/v) containing lithium bromide, which was dissolved to a concentration of 0.05 M. Each sample was dissolved in this solvent to make a concentration of 1 mg/ml, filtered using a 0.45 µm syringe filter, and injected for analysis. The analysis was performed for a period of 45 minutes after injecting 40 µL of the sample solution. Polysaccharide standards (pullulan) were used for the calibration of the unit for molar masses ranging from 180 g/mol to 708 000 g/mol.

Sample solutions (0.5 g/L) were made using reverse osmosis water for SLS, and a 0.5 M sodium hydroxide solution for KRT and SOD. These sample solutions were mixed with a Folin-Ciocalteu (FC) reagent, and a sodium carbonate solution. The absorbance of the solutions was read at 760 nm using a photo spectrometer. The phenolic hydroxyl group content was determined using a calibration curve obtained from vanillin solutions of known concentrations. This method was adapted from Areskog *et al.* (2010) [22].

2.2.3 Pre-oxidation and ammoxidation

Pre-oxidation and ammoxidation reactions were carried out using previously described experimental conditions [12]. The reactions were carried out in a 1 L Parr reactor. The reactor comprised of a heating jacket, a PID temperature controller and a pressure relief valve. The reaction conditions are shown in Table 1. The reagent (ammonia solution or hydrogen peroxide solution, for pre-oxidation or ammoxidation, respectively) was diluted to the desired concentration inside a fume hood. The sample was then loaded into the reactor, followed by the reagent solution. The reactor was then sealed, pressurized with air to 8 barg, and heated to the desired temperature (while stirring at 250 rpm). Air under pressure was used as the oxidant instead of pure oxygen, due to the explosion risk associated with its use, and higher operating cost compared to air. The heating time was approximately fifteen minutes. Upon completion of the reaction time, the heating jacket was removed, and a water bath was used to cool the reactor to 40 °C. The pressure relief valve was then opened to release pressure in the system, and the reaction mixture was collected, and spray dried to recover the products. Each raw material was pre-oxidized and ammoxidized in duplicate.

Table 1
Reaction conditions

Parameter	Value
Pre-oxidation	
Temperature	40 °C
Oxidant	Hydrogen peroxide
Time	4 hours
H ₂ O ₂ mass % mixture	1.5 and 5
Lignin mass % mixture	9
Stirrer speed	250 rpm
Ammoxidation	
Temperature	80 °C
Oxidant	Air
Pressure	10 barg
Time	4 hours
Ammonia % mixture	7
Lignin % mixture	10
Stirrer speed	250 rpm

2.2.4 N-lignin characterization

Each sample (0.25 g ± 0.01 g) was mixed with 250 ml reverse osmosis water and stirred at 250 rpm using a magnetic stirrer for fifteen minutes. The pH and electrical conductivity (EC) were then measured using a Eutech pH 6+ pH meter and a Crison CM 35+ electrical conductivity meter, respectively. The salt index was calculated as follows:

$$SI = \frac{EC_{\text{of sample solution}}}{EC_{\text{of sodium nitrate solution}}} \times 100\% \quad \text{Equation 1}$$

The elemental compositions of the synthesized N-lignins were determined according to the method described in section 2.2.2

2.2.5 Pot trial evaluation

The pot trial evaluation was conducted in a nursery at the Wood Science department, at Stellenbosch University. Stellenbosch is situated at an elevation of ~ 122 m above sea level. The midday temperature range in the nursery was 19 – 28 °C over the course of the trial.

2.2.6 Soil characterization

Malmesbury river sand was used for the plant trial evaluation of the N-lignins. The sand was characterized to provide insight into its nutrient composition, physical properties, and how the properties may affect plant growth. Table 2 summarizes the analytical methods used in the characterization of the sand.

Table 2
Soil characterization methods

Property	Method
pH	Potassium chloride extract
Total acidity	Potassium sulfate extract
Exchangeable K, Ca, Mg, Na	Ammonium acetate extract, read on ICP OES
Extractable B, Cu, Zn, and Mn	Ethylenediaminetetraacetic acid EDTA extract, read on ICP OES
Organic C	Walkeley-Black method
Extractable P	Citric acid extract, read on ICP OES
Nitrogen	Kjeldahl method, read on gallery
Field capacity	A sand sample (100 g) was saturated with water and allowed to stand for an hour, while the leachate was collected and measured. The volume of water that remained in the sample was determined by difference, and the filed capacity was calculated as the volume of water contained per unit mass of dry sand.

Plant pots (height = 12 cm, cross-sectional area = 176.71 cm²) were filled with 1500 g ± 1 g of Malmesbury river sand. Twenty-one seeds were planted in each pot, using a spacing of 2.5 cm. Each pot was irrigated with 50 ml tap water every two to three days. The seeds were allowed to germinate and grow for a period of 10 days. After 10 days, 18 seedlings which had a height of 40 – 50 mm were selected in each pot and allowed to grow further. The N-lignins were applied to the pots with irrigation water at a rate of 5 g/pot three times over a period of five days, amounting to a total of 15 g N-lignin per pot (1% of the soil mass) for each treatment. Each treatment was completed in triplicate. The pots were placed randomly in the nursery to avoid statistical bias. Phosphorus and potassium were supplied

through the application of mono-potassium phosphate (KHPO₄) at an application rate of 60 kg/ha and 76 kg/ha, respectively [23].

2.2.7 Biomass mass yield

The fresh mass yield was determined at the end of the four-week cultivation period. The seedlings were cut 2 mm above the soil surface and weighed to determine the fresh mass yield per pot. The fresh mass yield (t/ha) was then calculated using Equation 2:

$$\text{Fresh mass yield (t/ha)} = \frac{\text{Freshmassharvested (g / pot)}}{\text{Surfaceareaofpot (ha)}} \times \frac{1\text{ton}}{1000000\text{g}}$$

Equation 2

3 Results And Discussion

3.1 Raw material properties

The raw lignins were characterized by low nitrogen contents (0.2–0.3%) and high C/N ratios (177.6 to 281.5), supporting the need for nitrogen enrichment before their application as soil organic amendments (Table 3). Notably, SLS had a low carbon content (28.3%) due to the proportionally high ash content (61.14%) compared to KRT and SOD, and in comparison to lignins ammoxidized in previous studies (ash content ~ 20%, carbon content : 40–60%) [15, 16]. High ash contents and low carbon contents are not desirable because they are indicative of a lower quantity of organic substrate (lignin) into which nitrogen is bound during ammoxidation. In previous studies, the N-lignins still contained about 40% carbon even after ammoxidation [9]. Conversely, KRT and SOD had higher carbon contents and were within the range of lignins studied previously (40–60%) [15, 16, 23]. KRT (6.3%) and SLS (6.2%) had appreciable sulphur contents originating from the pulping chemicals, as opposed to SOD (0.3%) (Table 3). Further evidence for the presence of sulphur in these materials was provided by the appearance of a peak at 1112 – 1118 cm⁻¹ (6, sulphate) for KRT and SLS, which was not present in SOD (Table 3, Fig. 1). The presence of sulphur in the raw materials is a desirable characteristic because sulphur is necessary for amino acid synthesis in plants [25, 30].

Table 3
Proximate analysis of the starting lignins

	C [%]	N [%]	H [%]	S [%]	C/N	Ash [%]
KRT	56.3	0.2	6.5	6.3	281.5	0.5
SOD	56.4	0.3	7.4	0.3	188.0	0.75
SLS	28.3	0.2	3.6	6.2	176.6	61.14

Kraft lignin (KRT) was characterized by a higher quantity of phenolic groups (6.05 mmol/g) compared to SOD (3.22 mmol/g) and SLS (0.37 mmol/g) (Fig. 1, Table 4). Since the cleavage of inter-unit linkages during delignification results in the formation of phenolic low molecular weight fragments, it can be anticipated that harsher pulping processes such as Kraft processes will produce lignins with more free phenolic groups [29]. FTIR revealed an absorption band at 3400 cm⁻¹ (**1**) for KRT, which corresponded to the bending of phenolic groups (Fig. 1). This band was observed in both KRT and SOD but not in SLS, confirming the contents of phenolic groups in these samples, as observed from the UV analysis (Fig. 1, Table 4). Phenolic groups influence the reactivity of lignin to various reaction processes, including ammoxidation. About 60% of the nitrogen bound in lignin during ammoxidation is incorporated through the degradation of phenolic groups [32–35].

Table 4

Phenolic groups, molecular weights, total sugar of the starting lignins

Lignin	Phenolic group content (mmol/g)	Molecular weights (g/mol)			Total sugar (wt. %)
		Mn	Mw	Mw/Mn	
KRT	6.05	221	2563	11.60	14.21
SOD	3.22	169	3936	23.29	23.39
SLS	0.37	1842	3532	1.92	7.74

Furthermore, FTIR revealed that SLS did not contain an appreciable quantity of methoxyl groups, in contrast to KRT and SOD. This was deduced from the absence of peaks at 2900 cm⁻¹ (**2**, C-H stretching of methyl and methylene groups) and at 2820 cm⁻¹ (**3**, C-H vibrations of methoxyl groups) for SLS (Fig. 1). The absence of a peak at 1325-1330 cm⁻¹ (**5**, C-O of S ring) in SLS and at 835 cm⁻¹ (**7**, C-H out of plane in position 2 and 6 of S units) confirmed this observation. KRT originated from hardwood biomass, while SOD originated from sugarcane bagasse. Both these lignins were expected to contain an appreciable content of S units, which contain two methoxyl groups. Conversely, SLS originated from a mixture of hardwood and softwood. Softwoods do not contain appreciable quantities of S units, resulting in a lower abundance of S units in SLS. Due to their ease of oxidation, methoxyl groups are also vital for ammoxidation, as the amount of nitrogen incorporated into lignin increases linearly with the degradation of methoxyl groups [11, 31]. Based on the lower abundance of methoxyl groups and phenolic groups in SLS compared to KRT and SOD, it could be predicted that this raw material would incorporate a lower quantity of nitrogen during ammoxidation.

SOD had a higher sugar content (23.39%) compared to KRT (14.21%) and SLS (7.74%) (Table 4). FTIR revealed an appreciable absorption peak at 1709-1738 cm^{-1} (4) for SOD and KRT, in contrast to SLS (Fig. 1); this peak is attributed to the C=O stretch in unconjugated ketones and carbonyl groups which are present in carbohydrates [19]. SOD was obtained from sugarcane bagasse, a non-wood lignocellulose that has a high content of lignin-carbohydrate complexes, resulting in greater difficulty with the isolation of the lignin from carbohydrates (cellulose and hemicellulose), compared to woody lignocelluloses [17, 25, 31]. Kraft pulping is known to exert a greater extent of carbohydrate removal from biomass compared to NSSC pulping, resulting in a higher total sugar content in this raw material compared to NSSC [29].

The presence of monomeric sugars in lignin samples may result in the formation of considerable amounts of phytotoxic substances (>120 mg/g-extract) during ammoxidation at 100 °C. However, lower quantities were obtained (<16 mg/gextract) at 70 °C [30]. Thus, for the ammoxidation of sugar containing lignin at 80 °C (present study), the formation of toxic substances was deemed to not be a concern.

3.2 Elemental composition of N-lignins

The nitrogen contents of the synthesized N-lignins obtained from ammoxidation followed the expected reactivities of each starting material, based on the properties as reported earlier [9]. KRT had the highest reactivity based on the total nitrogen incorporated, while SLS was the least reactive. The high reactivity of KRT compared to SOD and SLS was related to its increased contents of free phenolic and methoxyl groups (Fig. 1, Table 4). The nitrogen content of KRT increased from 0.2% (KRT) to 2.8% (N-KRT) after ammoxidation (Table 5). This decreased the C/N ratio from 281.5 to 18.3, meeting the criteria for use as a soil amendment ($\text{C/N} < 20$). However, ammoxidation alone was not enough to decrease the C/N of both SOD (28) and SLS (70) to desirable levels, due to their decreased reactivities. Despite an improvement in the total nitrogen content from 0.30–1.90%, N-SOD had a high C/N ratio. SLS had a total nitrogen content of 0.4% after ammoxidation, and a C/N ratio of 70 (Table 5).

Table 5
Elemental composition of N-lignins

	C [%]	N [%]	H [%]	S [%]	C/N
KRT	56.30	0.20	6.50	6.30	281.5
N-KRT	51.20	2.80	6.70	5.10	18.29
N-O _{1.5} -KRT	46.20	3.20	7.10	5.70	14.44
N-O ₅ -KRT	49.48	4.02	6.15	5.64	12.31
SOD	56.40	0.30	7.40	0.30	188.00
N-SOD	53.20	1.90	7.40	0.40	28.00
N-O _{1.5} -SOD	51.70	2.10	7.60	1.80	24.62
N-O ₅ -SOD	52.82	3.34	6.64	0.92	15.81
SLS	28.30	0.20	3.60	6.20	141.50
N-SLS	28.00	0.40	3.50	8.78	70.00
N-O _{1.5} -SLS	24.00	0.50	3.20	5.70	48.00
N-O ₅ -SLS	23.57	1.61	2.73	6.31	14.64

The industrial lignins isolated in the present study exhibited lower reactivities to ammoxidation compared to the commercial lignins reported previously. For example, Tyhoda (2008) [12] reported that N-lignins containing 3–6.25% nitrogen could be synthesized from commercial lignins through ambient pressure ammoxidation, while N-lignins containing 0.4–2.8% nitrogen were obtained in the present study. This difference in reactivity may be attributed to differences in functional group compositions between industrial lignins and commercial lignins. It has been shown previously that IndulinTM (commercial kraft lignin) had lower molecular weights and higher phenolic group content compared to industrial hardwood kraft lignin, while SucrolinTM (commercial bagasse lignin) contained more carboxyl groups and more oxygenated aliphatic structures compared to soda lignin from bagasse pulping [31]. Highly oxidized technical lignins are more reactive to ammoxidation [12]. Furthermore, the use of a Parr reactor with air as the oxidant in the present study may have not reached the extent of ammoxidation similar to that reached in an immersion jet setup or high pressure oxygen used in previous studies [15, 16, 23].

Since the N-lignins obtained in this study did not meet the criteria for use as soil organic amendments (C/N < 20; Table 5), it was necessary to pretreat the raw materials with either 1.5% or 5% hydrogen peroxide to improve the subsequent nitrogen enrichment through ammoxidation, especially for SLS and SOD (Table 5). Pre-oxidation degrades organic substrates and increases the number of oxygen rich

functional groups, through which nitrogen is bound, and thus increases the amount of nitrogen incorporated during ammoxidation [25].

Pre-oxidation with 1.5% hydrogen peroxide increased the amount of nitrogen incorporated into KRT by ~14.3–3.2% (N-O_{1.5}-KRT) yielding a C/N ratio of 14.44, while the amount of nitrogen incorporated into SOD increased by 10.5–2.1% and 24.62 C/N (N-O_{1.5}-SOD) (Table 5). Similar improvements in nitrogen incorporation through pre-oxidation have been reported previously. Tyhoda (2008) [12] reported that pre-treating a bagasse lignocellulosic residue with hydrogen peroxide improved the amount of nitrogen incorporated during ammoxidation by 16.7% from 2.64–3.08%. However, the lignins investigated in this study at the selected reaction conditions had already shown a lower reactivity to ammoxidation compared to lignins studied previously [15, 16, 23]. Consequently, pre-oxidation with 1.5% hydrogen peroxide was not sufficient to produce N-lignins with desirable C/N ratios from SOD and SLS. N-O_{1.5}-SOD had a C/N ratio of 24.62, and N-O_{1.5}-SLS still had a high C/N ratio of 48 (Table 5).

Employing a higher oxidant concentration (5% H₂O₂) in the pre-oxidation yielded N-lignins with C/N ratios less than 20 from all three raw materials. The N-lignins obtained from KRT, SOD, and SLS had nitrogen contents of 4.02%, 3.34% and 1.61%, respectively (Table 5). This corresponded to C/N ratios of 12.31, 15.81 and 14.64, respectively. Indeed, SLS had the lowest reactivity to ammoxidation due to a lower abundance of reactive functional groups (methoxyl and phenolic groups) (Table 4, Fig. 1). It was noted that SLS only incorporated 1.61% N after pre-oxidation with 5% hydrogen peroxide, while 1.9% N could be incorporated into SOD without pre-oxidation. However, a lower amount of nitrogen had to be incorporated into SLS to lower its C/N to desirable levels (C/N < 20), due to its low carbon content. Consequently, the C/N of SLS (14.64) was slightly lower than that of SOD (15.81) after pre-oxidation with 5% hydrogen peroxide, despite incorporating a lower nitrogen content (Table 5).

3.3 Acidity, electrical conductivity, and salinity index of N-lignins

Soil amendments can affect the soil pH and salinity levels, which affect plant nutrient uptake and the biological activities of soil microorganisms [1, 16]. A soil pH between pH 5 and pH 7 is beneficial for both soil microorganisms and plants [38, 39]. N-O₅-lignins obtained from KRT (pH 5.48) and SOD (pH 6.51) had lower pH values, while the N-O₅-lignin obtained from SLS (pH 8.81) had a high pH (Table 6). The application of N-O₅-KRT (pH 5.48) and N-O₅-SOD (pH 6.51) on neutral soil was thus not expected to change the soil pH beyond the optimum range (pH 5 to pH 7), as opposed to the application of N-O₅-SLS (pH 8.81) [32]. High soil pH may retard plant growth [34].

Table 6
pH, electrical conductivity, and salinity index of N-lignins

	pH	Electrical conductivity (dS/m)	Salinity index (%)
N-O₅-KRT	5.48	0.591	16.53
N-O₅-SOD	6.51	0.369	10.32
N-O₅-SLS	8.81	2.27	63.62

N-O₅-KRT (0.591 dS/m, 16.53% SI) and N-O₅-SOD (0.369 dS/m, 10.32% SI) had considerably lower electrical conductivities (EC) and salinity indexes (SI) compared to N-O₅-SLS (2.27 dS/m, 63.62% SI) (Table 6). N-O₅-KRT and N-O₅-SOD were anticipated to have lower salt indexes compared to N-O₅-SLS due to a lower ash content in the starting materials (Table 3, Table 6). The salt index of N-O₅-SLS was considerably high even compared to commercial fertilizers such as ammonium sulfate (68.3%) and potassium sulfate (42.6%) [35]. A high salt concentration in the soil solution can retard plant growth through increasing the soil osmotic pressure, depriving plants of water, and through causing ion competition with essential plant nutrients ($\text{Na}^+/\text{Ca}^{2+}$, Na^+/K^+ , and $\text{Cl}^-/\text{NO}_3^-$) [36]. Most vegetables have a salinity threshold of 1 to 2.5 dS/m in saturated soil extracts [37]. N-O₅-SLS had an EC that is on the upper end of this range and may cause growth inhibition to sensitive plants (Table 6) [42, 44].

3.4 Evaluation on crops

The ammoxidation (after pre-oxidation for some) of all three starting materials yielded products that had C/N ratios similar to that of humified soil (10 – 15), indicating their suitability for use as soil amendments (Table 5). However, previous studies have shown that N-lignins may have a negative impact on plant growth due to toxic substances formed during ammoxidation. Lignosulfonates are also susceptible to lumping and crust formation on the soil surface, which hinders the growth of new seedlings, due to their high solubility [9, 12]. It is thus necessary to assess these products in a plant trial, even though they meet the C/N criteria (< 20). To test the suitability of these N-lignins, a plant trial was conducted using white mustard (*Sinapis Alba*).

3.5 Sand properties

The properties of Malmesbury sand are shown in Table 7, and were compared to the critical levels for nutrient deficiency as reported by Ngezimana and Agenbag (2014) [32]. The pH (5.9) of the soil was within the optimal range for plant growth: pH 5 to pH 7 [32] (Table 7). The application of N-O₅-KRT (pH 5.48) and N-O₅-SOD (pH 6.51) may not cause considerable changes to the soil pH compared to N-O₅-SLS (pH 8.81), which had a considerable difference to the pH of the sand (2.9 pH units) (Table 7). The macro- and micronutrient contents of the soil were below the critical levels for nutrient deficiency for all nutrients except for copper (actual level: 0.71 mg/kg, critical level: < 0.3 mg/kg) and zinc (actual level: 0.65 mg/kg, critical level: < 0.5 mg/kg), while the sodium content was below toxic levels (actual level: 7 mg/kg, critical

level: > 250 mg/kg) (Table 7). The low nutrient and organic carbon (0.07%) content status of the sand was a desirable characteristic because the nutrient status of the sand would not interfere with the results of the trial. The field capacity (20 ml/100 g dry sand) was sufficiently high to avoid leaching for an irrigation rate of 50 ml/1500 g sand every two to three days (Table 7).

Table 7
Properties of Malmesbury sand

Property	Unit	Value	Critical level
pH	5.9		< 5 (optimal: pH 5 to pH 7)
N	Not detected		-
Calcium	cmol(+)/kg	0.42	< 1.0
Magnesium	cmol(+)/kg	0.07	< 0.4
Potassium	mg/kg	10	< 60
Sodium	mg/kg	7	> 250
Phosphorus	mg/kg	7	< 36
Total cations	cmol(+)/kg	0.56	-
Copper	mg/kg	0.71	< 0.3
Zinc	mg/kg	0.65	< 0.5
Manganese	mg/kg	0.39	< 5
Boron	mg/kg	0.02	< 0.2
Carbon	%	0.07	-
Sulphur	mg/kg	2.63	< 6.0
Iron	mg/kg	10.98	-
Field capacity	ml/ 100 g-dry sand	20 ± 3.2	-

3.6 Effect of the N-lignins on plant growth

N-O₅-KRT (3.5 t/ha) and N-O₅-SOD (3.21 t/ha) increased the fresh mass yield of seedlings by 71.6% and 57.4%, respectively, when compared to the control (Fig. 2, Fig. 3). The yields obtained with these N-lignins were equal to 78% and 85% of the yield obtained with the commercial fertilizer, respectively. N-lignins are primarily soil conditioning materials, and are expected to have lower yields compared to commercial fertilizers due to their lower nitrogen content [14, 20, 23]. However, these materials proved to be suitable for use as soil conditioning materials (C/N < 20), as evidenced by improved crop yield, indicating that

some of the nitrogen bound onto these materials was available for plant uptake over the four-week cultivation period. Conversely, the seedlings treated with N-O₅-SLS withered within one week after the treatment (the yield shown in Fig. 2 is derived from the remains of the seedlings and not fresh mass obtained). This was ascribed to the high pH and salt index of this material (Table 6), which limits its use as an organic soil amendment [32].

4 Conclusions

This study demonstrated that the investigated lignins (KRT, SOD, and SLS) could be ammoxidized to produce N-lignins with C/N ratio less than 20. However, this could not be accomplished with SOD and SLS without pre-oxidation with hydrogen peroxide (5%). The reactivity of these materials could be ranked as follows; KRT > SOD > SLS. The difference in the reactivity of the selected lignins was due to the differences in the molecular structure of these materials, especially phenolic and methoxyl group composition. A pot trial assessment revealed that N-lignins obtained from KRT and SOD were non-toxic and improved the fresh mass yield of seedlings. N-lignins obtained from SLS resulted in crop failure, which was attributed to the high pH and high salt index of this material. Further studies may assess the profitability of the industrial production of N-lignins from kraft and soda lignins, conduct long term studies on the application of N-lignins as soil rehabilitation materials to assess their slow release nitrogen characteristic, and assess the suitability of these N-lignins for use in different soil types and plant types.

Declarations

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Availability of data and material:

The resulting data from the study are available from the corresponding author upon request.

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Figures

Figure 1

FTIR spectra of the starting lignins

Figure 2

Fresh mass yield of seedlings

Figure 3

White mustard seedlings fertilized with ammoxidized lignins, 4 weeks after fertilization; N-05-SLS, N-05-SOD, N-05-KRT, No fertilizer, and the Commercial reference

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