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Research

Dendrochemical analysis of *Brachystegia longifolia* Benth as an indicator of Sulphur deposition in a copper smelting area of Mufulira, Zambia

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Abstract
High levels of Sulphur dioxide (SO_2) emanating from a copper smelter in Mufulira, Zambia has been reported around the year 2000, exceeding environmental quality guidelines. This resulted in the overhauling and upgrading of an obsolete smelter, the principal source of pollution. However, there is limited environmental evidence on the comparative variations in SO_2 emissions in Mufulira before and after the smelter upgrade. This study investigated the dendrochemical potential of *Brachystegia longifolia* in detecting environmental change induced by variations in industrial pollution load. *B. longifolia* wood discs were extracted at breast height, air-dried and sanded until the transverse surface and annual rings were clearly visible. µ-XRF spectrometer was used to determine the distribution of Sulphur by scanning and exposing samples along the bark-pith transverse surface to the X-ray beam, obtaining X-ray transmission intensity values of counts per second (cps) for Sulphur. Finally, the same radial slices were then scanned with a cellulose acetate calibration scale using an X-ray densitometry chamber. Digital images were then analysed with Windendro® program to determine wood density. The mean Sulphur distribution in tree rings at the beginning (2007) and completion (2014) of the smelter upgrades were 15.41 cps and 17.51 cps, respectively. By 2019, the highest Sulphur content in *B. longifolia* wood had significantly ($p < 0.05$) reduced to 13.28 cps. Furthermore, a significant reduction in wood density with increasing tree age was observed after the installation of the new smelter. 0.78 g/cm^3 and 0.75 g/cm^3 mean wood density were recorded in annual rings at the beginning and completion of the upgrades, respectively. However, the mean wood density had significantly ($p < 0.05$) reduced to 0.67 g/cm^3 by 2019. The significant variations in the distribution of Sulphur and wood density in tree rings formed before and after upgrading an obsolete copper smelter demonstrates the dendrochemical potential of *B. longifolia* and possibly other Miombo woodland species, offering an opportunity to biomonitor the impact of industrial activities in the Miombo landscapes which are extensively distributed across sub-Saharan Africa.

Article Highlights

- Mining processes emit potentially toxic elements, including SO_2 that are dispersed across nearby landscapes.

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- Our study demonstrates that *B. longifolia* absorbs and sequesters Sulphur in a trend that reflects the generation of SO_2 from a point source.
- These results suggest that *B. longifolia* and possibly other miombo species have the potential to biomark and bio-monitor environmental change.

Keywords Copper smelter · Sulphur dioxide · *Brachystegia longifolia* · Annual rings · Sulphur distribution · Wood density

1 Background

Mufulira town (Zambia) is one of the most polluted mining towns in the World [1]. A number of reports [2, 3] highlight the high levels of Sulphur dioxide (SO_2) around the year 2000 emanating from a copper smelter located in Kankoyo township dispersed across the nearby landscape, exceeding environmental quality guidelines-EQG. This resulted in overhauling and upgrading the smelter in 2007 and by 2014, the facility had been upgraded to a stage where it could capture 97% of SO_2 produced at the facility [3], converting it to Sulphuric acid-H_2SO_4. However, there is limited environmental evidence on the comparative variations in SO_2 emissions in Mufulira before and after the smelter upgrade.

Mineral beneficiation processes in the mining industry emit flue gas and particulate matter comprising SO_2, among other toxic substances [4]. Wind dispersion aids the mobilization of these potentially toxic elements (PTEs) from mining operations into the environment and leads to the contamination of air, surface soil and water bodies [5, 6]. Some plants take up these PTEs in excess, resulting in deleterious effects on the health and productivity of vegetation [7, 8].

SO_2 enters plant tissues through the lenticels, leaves, and roots [9]. In the plant tissues (mesophyll cells), it reacts with water molecules and converts to Sulphate (SO_4^{2-}) or Sulphite (SO_3^{2-}) ions [10, 11]. However, excess $SO^{2-}{}_3$ is highly toxic to plants, affecting plant growth and productivity [12]. The detoxification process of SO_3^{2-} to SO_4^{2-} increases the generation of reactive oxygen species (ROS) such as superoxide radicals ($O_2^{\cdot-}$) and hydrogen peroxide-H_2O_2 [13]. In addition, stomatal closure triggers an insufficient concentration of intercellular CO_2, instigating the formation of singlet atoms ($^1O_2$) and hydroxyl radicals ($OH^\cdot$), other family members of destructive ROS [14–16]. According to Mansoor et al. [14], ROS are highly toxic and destructive to biomolecules such as lipids, proteins, nucleic acids, and carbohydrates.

$^1O_2$ severely damages nucleic acids, proteins, pigments, and lipids [17], jeopardizing photosynthetic and photosystems machinery [16]. $O_2^{\cdot-}$ transforms into the more reactive and toxic $OH^\cdot$ and $^1O_2$, causing lipid peroxidation [18]. $OH^\cdot$ is the most toxic and reactive among the members of the ROS family [15], destroying cellular compartments through, lipid peroxidation, membrane damage and protein destruction [17, 18]. Zentgraf et al. [19] noted that the longer half-life of H_2O_2 enables it to traverse long distances and cross cell membranes through aquaporins, covering significant lengths within the cell and causing oxidative damage. They further argued that H_2O_2 is responsible for programmed cell death and 50% loss of enzymatic activities at a high cellular concentration. Therefore, extensive SO_2 exposure affects morphological and biochemical activities resulting in reduced plant growth and forest productivity [20].

Field-based studies have investigated the potential of using SO_2 and plants in detecting industrial pollution-instigated environmental change by evaluating the concentration of Sulphur in different tree species and its associated effect on tree growth and wood cell characteristics [21, 22]. Sensula [23] observed an increased concentration of Sulphur in *Pinus sylvestris* growing near industrial areas and reported reduced annual ring width attributed to SO_2. In addition, Likus-Cieslik et al. [21] recorded a higher concentration of Sulphur in the leaves of *P. sylvestris* growing near an industrial site and reported a significant correlation between the concentration of Sulphur in needles and stand defoliation degrees.

Thus far, there is limited information on the dendrochemical potential of Miombo woodland species in detecting changes in industrial pollution. This study seeks to unravel the dendrochemical potential of *Brachystegia longifolia* in detecting environmental change induced by variations in industrial copper mining activities around the Miombo woodlands. This was achieved through (i) determining the Sulphur concentration in tree rings, representing annual deposition of SO_2 in *B. longifolia*, (ii) dating the Sulphur concentration in annual tree rings, (iii) identifying variations in SO_2 deposition before and after the installation of a modern smelter and iv) determining the effect of SO_2 variations on the wood density of *B. longifolia*.

Characterized by Julbernardia, Isoberlinia and Brachystegia species, Miombo woodlands extend widely in the Sub-Sahara Africa covering 2.7 million km^2 of Africa's landmass, representing 9% of its total area [24]. This forest type is extensively distributed in Angola, Mozambique, Malawi, the Democratic Republic of Congo, Zambia, Zimbabwe and Tanzania. Most of these countries are endowed with mineral resources where mineral extraction and beneficiation activities have been carried out commercially for close to a century. In Zambia, at least 80% of forest cover is classified as Miombo woodland [25]. However, the potential impacts of mining activities on the Miombo woodland species and forest productivity are not well established.

2 Materials and methods

2.1 Study area description

Mufulira is a copper mining town in Zambia, highly polluted with SO_2 [3] and copper [26]. The principal source of pollution is a copper smelter (Fig. 1) located in Kankoyo township with its associated facilities such as the converter slag blow, converter copper blow, and matte setting furnace [27].

Commissioned in 1936, Mufulira smelter is the oldest and largest in Zambia and treats concentrates from the Copperbelt region, Northwestern Zambia, and in-house mining operations [26]. This facility was replaced with a modern Isasmelter in 2014 [28]. The new smelter converts more than 97% of SO_2 produced at this mining facility into H_2SO_4. However, there is limited environmental evidence on the comparative variations in SO_2 emissions in Mufulira before and after the smelter upgrade. Therefore, this study investigated the impact of copper mining activities on the Miombo woodlands by assessing the potential of *B. longifolia* in detecting industrial pollution dynamics through dendrochemistry and analyzing Sulphur distribution in tree rings formed before and after the replacement of the obsolete smelter. *B. longifolia* was preferred among the characteristic Miombo species because of previous studies demonstrating that *Brachystegia* species form annual growth rings [29], which is critical in Dendrochronological studies.

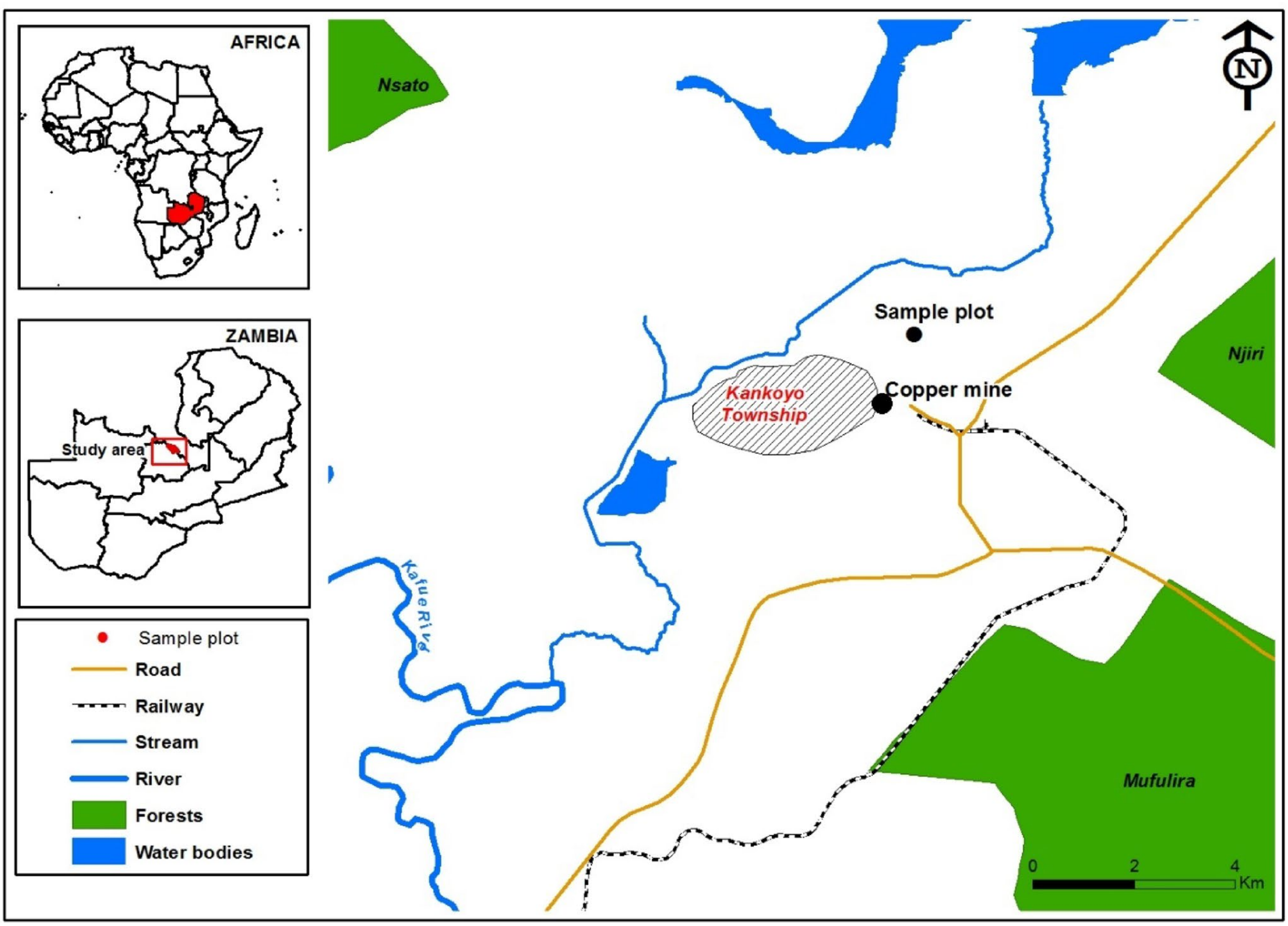

Fig. 1 Study area Map showing the location of the copper smelter and the sampling plot in Mufulira, Zambia

The study area recorded 21 °C mean annual temperature and 1147 mm mean annual precipitation between 1960 and 2016 [30].

2.2 Sampling and justification

Wood discs were collected from two *B. longifolia* trees (NNA and NNC) naturally growing from the nearest forest patch, 1.8 km from the copper smelter (Fig. 1). Each disc then produced two samples (NNA-A, NNA-B, NNC-A and NNC-B) for Sulphur and wood density analysis, giving a total of four samples. The limited number of sampled trees was because of the destructive nature of the sampling method. Furthermore, Cherubini et al. [31] and Mifsud et al. [32] guided that sample location and sample extraction height along the tree trunk as well as the number of samples per tree are the most important parameters to consider when sampling for dendrochemical studies. This is because the correlation between environmental change drivers and elemental concentrations in tree rings offers great annually and intra-annually resolved proxy potential as the average concentration of the studied element(s) is drawn from hundreds of data points for a single year depending on the width of the growth band/ring (Fig. 2) and trees are statically available overtime [31, 33–35].

Therefore, studies [35–38] that have sampled discs using destructive sampling for dendrochemistry usually aim at sampling the bare minimum to reduce a contribution to deforestation. Cobanoglu et al. [37], Scharnweber et al. [35] and Battipaglia & Cherubini [36] sampled one tree each to study the effect of traffic density and mineral extraction on the concentration of trace elements in tree rings. Conversely, Fornasaro et al. [38] and Luong et al. [39] sampled three trees for similar objectives, without any reported significant differences in the overall results between and among studies with different sample sizes.

2.3 Sample preparation and analysis of Sulphur distribution and wood density in tree rings

Sample preparation: Wood discs were extracted at breast height (≈1.3 m) of the selected trees (Fig. 2), air dried and sanded progressively using 80 to 600 grits until the transverse surface and annual rings were clearly visible. Two radial samples of each disc were cut for the analysis of Sulphur and wood density. The radial samples were glued on a wood support and slides were cut transversely (1.7 mm thick) with a double circular saw. Radial wood slices were then kept at 20 °C and 60% relative humidity until reaching a stable moisture content of 12% (~ 24 h) [40].

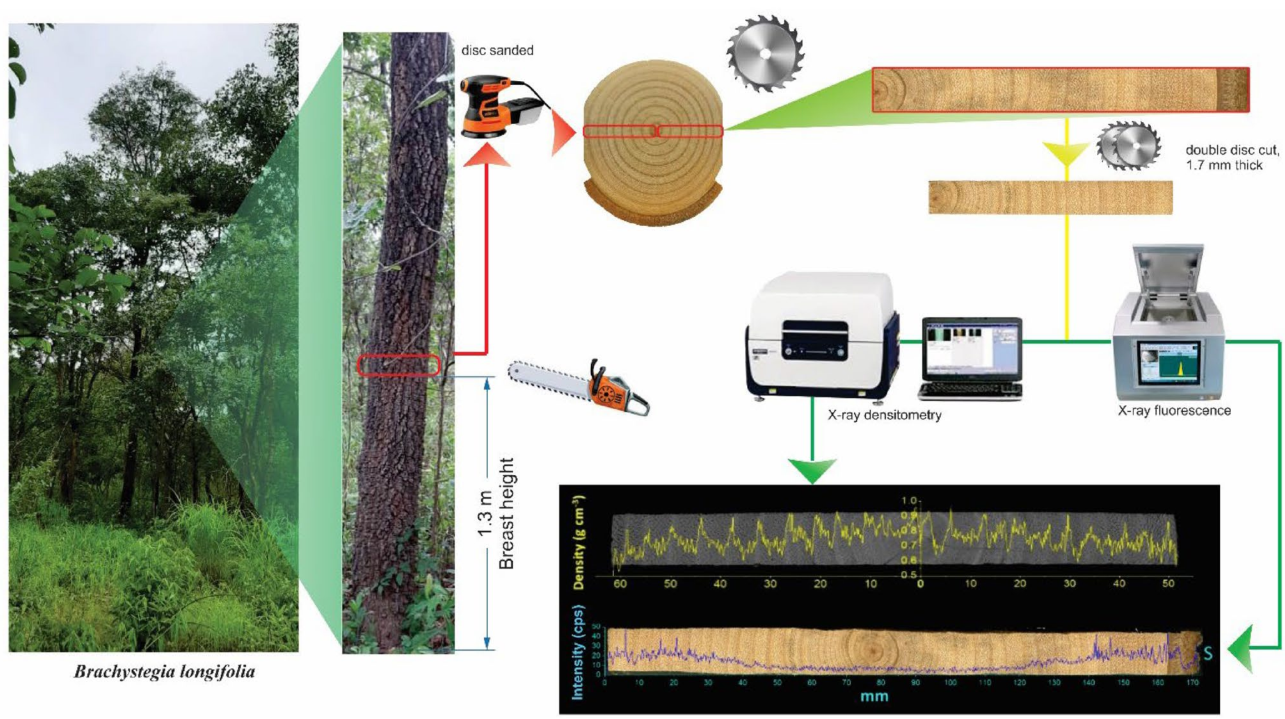

Fig. 2 Sample preparation and analysis of Sulphur distribution and wood density in *B. longifolia* trees

Sulphur distribution analysis: Radial slices were linearly scanned along the bark-pith transverse surface. Analyses were performed with a µ-XRF spectrometer (Bruker, M4 Tornado, USA), with an Rh tube (50 kV, 600 µA) and a silicon drift detector. Samples were exposed to the X-ray beam with 25 µm width for 100 ms at each measurement point (dead time of 15%) in the radial direction (25 µm step intervals) and in vacuum condition (< 0.5 Torr). X-ray transmission intensity values of counts per second (cps) for Sulphur were analysed to distinguish the detected signal from the background with reasonable certainty for the analytical quantification and the Sulphur profiles in cps were finally built.

Wood density analysis: The same radial slices were then scanned with a cellulose acetate calibration scale using an X-ray densitometry chamber (Faxitron MX20-DC12, Faxitron X-Ray, IL, USA) [41]. Digital images (TIFF, 513 dpi) were analysed with the Windendro® program, obtaining wood density profiles in g/cm^3 with 15 µm step intervals. The position of the latewood-earlywood boundary was defined by Mulenga et al. [29]. Then annual time series of average Sulphur and wood density for each radial sample were produced.

2.4 Dating of Sulphur distribution and wood density

The sampling year (November 2020) served as the basis for dating tree rings. Annual growth rings were counted and the latest complete growth band was allocated to the previous full growth season (2019 winter plus summer), before the sampling year. Dating of wood samples was carried out systematically starting from the bark towards the pith. Thereafter, Sulphur distribution and wood density in *B. longifolia* trees were dated by allocating respective values according to the year when that tree ring was formed.

2.5 Statistical analysis

A simple linear regression analysis was conducted to determine whether the average wood density and average Sulphur concentration in tree rings significantly varied with time, after satisfying the requirements for a regression test. The test was carried out in two segments. The first analysed data before 2014 while the second one was for data after 2014. This was done to compare trends and the associated significance levels at $p < 0.05$ between the two time periods. All the graphics and statistics were produced using an Integrated Development Environment for R (RStudio IDE) which is a programming language for statistical computing and graphics [42].

3 Results and discussion

3.1 Sulphur distribution in *B. longifolia* tree rings

The number of annual growth rings demonstrated that the studied trees were aged 25 and 11 years old. Trees NNA and NNC started growing in 1994 and 2008, respectively and were both sampled at the same time in November 2020. The mean concentration of Sulphur in tree rings that developed in 2014 and 2019 were 17.51 cps and 13.28 cps, respectively (Table 1).

The distribution of Sulphur in tree rings formed before 2014 increased significantly ($p < 0.05$) with tree age or time (Fig. 3). Conversely, significant ($p < 0.05$) reduction of Sulphur concentration in *B. longifolia* tree rings was observed after 2014. These trends suggest significant differences in the concentration of Sulphur or Sulphur compounds in the atmosphere or soil before and after 2014. This can be attributed to the SO_2 emissions from the copper smelter as this is the only smelter within the radius of approximately, 30 km. Chambishi copper smelter is the nearest located in the downwind

Table 1 Concentration of Sulphur (cps) in *B. longifolia* tree rings in 2014 and 2019

Sample ID	2014	2019
NNC_A	15.14	14.01
NNC_B	12.52	14.48
NNA_A	22.26	16.64
NNA_B	20.11	8.00
Mean	**17.51**	**13.28**

Bold shows the average sulphur content and wood density, respectively

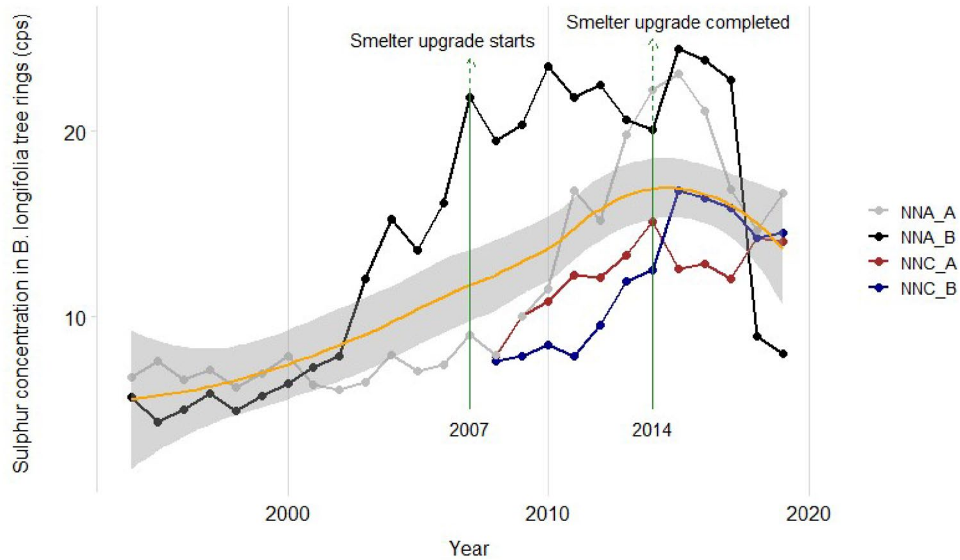

Fig. 3 Dated mean Sulphur distribution in *B. longifolia* trees naturally growing near a copper smelter in Mufulira, Zambia. The dating provides significant milestones for the copper smelter upgrade project

direction. Muma et al. [3], reported that SO_2 and heavy metals from stack emissions at the copper mine heavily pollute Mufulira and the surrounding landscapes. SO_2 levels exceeding the acceptable limit of 50 µg/m^3 annual exposure were reported within a radius of 12 km downwind and 4 km upwind. These exposure levels far exceed the EQG to protect fauna and flora.

The project to replace and upgrade a copper smelter aimed at minimizing environmental footprint by abetting the dispersion of SO_2 emissions into the atmosphere was completed around this period [28]. Briefly, the smelter upgrade project started in 2007 and was completed in 2014 [28]. The facility was by 2009 able to capture up to 51% of SO_2 from the mineral beneficiation processes and up to 97% at the completion of the project in 2014 [3, 28]. Therefore, the reduction of Sulphur distribution and trend in *B. longifolia* tree rings after 2014 can be attributed to a decrease in SO_2 emissions. Conversely, the increasing trend of Sulphur in tree rings before 2014 could have been instigated by increasing SO_2 dispersion triggered by increased copper production [43].

The mean Sulphur concentration in tree rings at the beginning and completion of the smelter upgrades were 15.41 cps and 17.51 cps, respectively. By 2019, the mean Sulphur content in *B. longifolia* wood had significantly reduced to 13.28 cps. A slight lag and downward trend in the distribution of Sulphur after the completion of the smelter upgrade can be attributed to residual (carry-over) Sulphur in the soil which was ultimately absorbed through the roots in the subsequent years.

Muma et al. [3] argued that although the smelting activities increased in the last two decades, atmospheric SO_2 recorded at approximately 1 km downwind of the smelter, reduced significantly between 2014 and 2018 from 247 to 105 µg/m^3. It would be expected that if the production capacity of the smelter increased after the upgrades, then SO_2 emissions would increase as well, potentially increasing the concentration of Sulphur in tree rings. In contrast, Muma et al. [3] observed decreased levels of atmospheric SO_2 a kilometre away, downwind of the copper smelter. Our study shows that the SO_2 footprint in tree rings is reduced, suggesting that a significant amount of this pollutant is being effectively captured by the modern smelter. Therefore, its emission into the atmosphere has significantly reduced, achieving the aim of the copper mining operator, which was to reduce the emission of SO_2 by converting it into H_2SO_4 and capturing it for industrial use [43].

Similar results have been reported elsewhere in different plant species, including *P. monophyla* [44], *P. pinea* [45], *P. elderica* [46], *Lax occidentalis* [47], *Picea abies* and *P. sylvestris* [48], and *Quercus pubescens* [49]. These species recorded increased concentrations of Sulphur in different biomass samples including tree rings at the peaks of SO_2 emanating from varying industrial activities.

3.2 Wood density in *B. longifolia* tree rings

On average, 0.78 g/cm^3 and 0.75 g/cm^3 wood density were recorded in annual rings at the beginning and completion of the upgrades, respectively (Table 2). However, the mean wood density had significantly reduced to 0.67 g/cm^3 by 2019.

Table 2 Density (g/cm3) of *B. longifolia* wood formed in 2014 and 2019

Sample ID	2014	2019
NNC_A	0.71	0.67
NNC_B	0.71	0.61
NNA_A	0.84	0.74
NNA_B	0.72	0.66
Mean	**0.75**	**0.67**

Bold shows the average sulphur content and wood density, respectively

A significant ($p < 0.05$) increase in wood density was observed in tree NNA with increasing age, before 2014 and thereafter decreased, significantly ($p < 0.05$), Fig. 4. Conversely, wood density for samples extracted from tree NNC decreased significantly ($p < 0.05$) before and after 2014.

Wood density increased significantly with increasing tree age before the upgrading of the smelter in tree NNA (Fig. 4). Conversely, a significant reduction in wood density with increasing tree age was observed from 2009 when the smelter upgrades could capture 51% of SO_2. This downward trend in wood density is observed in both trees, NNA and NNC. The observed changes in the density of *B. longifolia* trees before and after the upgrading of the copper smelter can be attributed to variations in the copper mining-induced toxic emissions. Emissions such as SO_2 may induce variations in tree growth rate [21, 22, 29], resulting in the changes in wood density of *B. longifolia* trees [29, 50–54].

A number of studies have evaluated the effect of tree growth rate on the wood density of several tree species including *Quercus petraea* [51] and *Acacia auriculiformis* [53]. All these studies reported a gradually decreasing wood density with an increasing tree growth rate. A higher growth rate induces increased cell lumen dimensions and increased cell wall thickness [55–57] critical for wood density. Werdin et al. [58] reported that wood density is significantly influenced by wood cell characteristics. They observed higher wood density in species with thinner cell walls and greater fibre diameters but recorded lower density in those with thicker cell walls and smaller fibre diameters. Therefore, the observed wood density in these studies was attributed to increased cell lumen diameters with significantly increased cell wall thickness formed at a higher growth rate.

Other studies have examined the impact of SO_2 phytotoxicity and reported significant increments in wood cell lumen diameter and reduced cell wall thickness in *Calendula officinalis* [59], *Pinus elderica* [46], *Mangifera indica* [60], *Prosopis cineraria* [61], *Syzygium cumini* [61], and *Ailanthus excelsa* [62].

Therefore, the observed changes in wood density before the smelter upgrades in our site can be attributed to the low tree growth rate instigated by copper mining-induced SO_2 phytotoxicity. Conversely, the reduction in wood density after 51% and 97% capture of SO_2 can be attributed to increased tree growth. It is assumed that the growth rate picks up

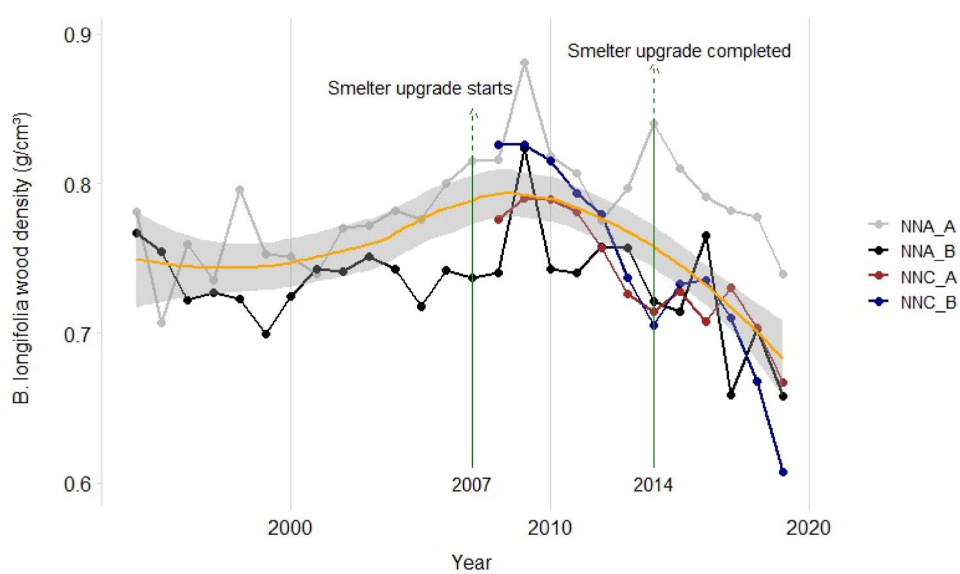

Fig. 4 Dated mean wood density of *B. longifolia* prior to and after replacement and upgrading of the copper smelter. The dating provides significant milestones for the copper smelter upgrade project

resulting in the formation of reduced cell lumen diameters and increased cell wall thickness after the significant capture of SO_2, leading to the demonstrated reduction in the density of *B. longifolia* wood (Fig. 4).

3.3 Study implication on the utilization potential of *B. longifolia* wood formed in a SO_2 polluted environments

B. longifolia is one of the most commercially valuable Miombo woodland species in Zambia and Southern Africa. Species of the genera *Brachystegia*, *Isoberlinia,* and *Julbernardia* are characteristic trees of the Miombo [63] and extensively spread across all mining countries, including Zambia, Angola, Malawi, the Democratic Republic of Congo (DRC), Tanzania, Zimbabwe, and Mozambique [24]. These tree species are widely used for furniture manufacturing, housing construction and bioenergy production [64]. The Miombo woodland is the most extensive vegetation type in Zambia covering over 80% of forests and accounts for 60% of Zambia's land mass [25]. This vegetation type covers 9% of Africa's total land area, covering over 2.7 million km^2 [65].

In Zambia, most mining activities are surrounded by forests characterized by Miombo species. Given the impact of environmental pollution on tree growth and forest productivity [21, 22, 29], there is a need to examine and exploit the biomass value chain to maximize the utilization potential of forests to sustain the bioeconomy. Potent economic opportunities are feasible for high-value products including bioenergy products, biopolymers, biocomposites, pulp, and chemical products. However, the utilization potential of biomass resources depends on characteristics such as density, chemical composition, machinability, and mechanical properties [66]. Therefore, the variations in wood density and Sulphur concentration observed in this study suggest that the utilization potential of *B. longifolia* trees and possibly other Miombo woodland species naturally growing in mining environments is limited.

Generally, this study suggests opportunities for multidisciplinary efforts exploring how to exploit and maximize the utilization potential of biomass generated from polluted environments, considering the expected high ash content instigated by high concentrations of Sulphur as demonstrated in this study. For instance, contaminated biomass with high-quality cellulose can be used to produce liquid-based biofuels [67, 68] and then utilize biomass remnants to manufacture lignocellulosic composites, among other innovative bio-based products. These multidisciplinary collaborations would unlock viable economic opportunities through the utilization of biomass produced in polluted environments advancing the cause of a circular economy.

Additionally, this study demonstrates that it is technically feasible for mining firms to take initiatives that minimize and mitigate the impact of mining activities on the environment. Furthermore, our study shows that the Miombo woodlands are vulnerable to environmental stresses instigated by anthropogenic activities such as mining and other industrial activities.

4 Conclusion

This study investigated the dendrochemical potential of *B. longifolia* in detecting environmental change induced by variations in industrial pollution load. The results show a significant ($p < 0.05$) reduction in the distribution of Sulphur after the completion of smelter upgrades. The mean Sulphur concentration in tree rings at the beginning (2007) and completion (2014) of the smelter upgrades were 15.41 cps and 17.51 cps, respectively. By 2019, the highest Sulphur content in *B. longifolia* wood had significantly reduced to 13.28 cps. Furthermore, a significant reduction in wood density with increasing tree age was observed after the installation of the new smelter. 0.78 g/cm^3 and 0.75 g/cm^3 mean wood density were recorded in annual rings at the beginning and completion of the upgrades, respectively. However, the mean wood density had significantly reduced to 0.67 g/cm^3 by 2019. The significant variations in the distribution of Sulphur and wood density in tree rings formed before and after upgrading an obsolete copper smelter demonstrates the dendrochemical potential of *B. longifolia* and possibly other miombo woodland species, offering an opportunity to biomonitor the impact of industrial activities in the miombo landscapes which are extensively distributed across sub-Saharan Africa.

Author contributions C.M. and C.A.L conceptualized the study and collected samples. D.R.O.R and HDB carried out laboratory sample analysis. C.M, D.R.O.R, HDB, D.P, C.A.L and J.H.S designed the methodology. S.A.M. designed Fig. 3. D.P. designed Fig. 1. C.M, D.R.O.R, H.D.B, D.P, C.A.L and J.H.S analyzed data and interpreted the results. C.M, DROR, HDB, D.P, C.A.L and J.H.S drafted the manuscript. JHS edited the final manuscript. All authors reviewed the manuscript.

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Data availability The data used during the current study available from the corresponding author on reasonable request.

Declarations

Ethics approval and consent to participate Not applicable.

Plant guidelines The authors confirm that the use of plants in the present study complies with international, national, and/or institutional guidelines.

Wild plants *Brachystegia longifolia* Benth was collected in Zambia by Royal Botanical Gardens. The plant material was identified by Boume. R, and a voucher specimen was deposited at Royal Botanic Gardens, Kew—Herbarium Specimens with ID K Herbarium K000417827.

Consent for publication Not applicable.

Competing interests The authors declare no competing interests.

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