

**Peer-Review Report for IJECC_121224:
INFLUENCE OF EDAPHIC FACTORS ON SULFUR
CONTENT IN *Calophyllum inophyllum* L. BIODIESEL**

Novelty of the Work

The study contributes valuable data on the sulfur content of biodiesel from *Calophyllum inophyllum* L., particularly with soil sulfur content. This adds to the knowledge of biodiesel quality control, especially for non-edible oil sources. However, the novelty could be strengthened by comparing the findings with other non-edible feedstocks or exploring the environmental implications in greater detail.

Specific improvements to be done to the manuscript:

- **Highlight Unique Contributions:** Emphasise how your work differs from previous studies. If there's a lack of studies focusing specifically on the sulfur content in *Calophyllum* biodiesel, this should be highlighted as a unique aspect of your research.
- **Broader Context:** Consider discussing the broader implications of your findings, such as the impact on biodiesel regulation, environmental impact, and energy sustainability.

Rating and Recommendation

- **Rating: 75/100**
- **Recommendation:** The manuscript demonstrates solid research and a clear presentation of data. However, it requires improvements in clarity, organisation, and depth of discussion. With these revisions, the manuscript can potentially contribute to the field of biofuels synthesis. I recommend a **major revision** before considering acceptance for publication. The specific recommendations are provided as follows.

1. Abstract

Summary: The abstract provides a brief overview of the study's aims, methodology, results, and conclusions. It highlights the investigation of sulfur content in Calophyllum biodiesel concerning soil sulfur content across different regions in Southern Karnataka.

Strengths:

- Clear identification of study areas and the purpose of the research.
- Mention of methodologies used, such as ICP-OES and turbidimetry.

Areas for Improvement:

- The abstract could benefit from a more specific description of the findings, particularly the comparative analysis of sulfur content in different regions.
- Quantitative data (e.g., sulfur content levels) should be presented to give a clearer picture of the study's findings.
- The conclusion could be more concise, focusing on the main takeaway rather than mentioning the need for further research.

Specific improvements to be done to the manuscript:

- **Objective Statement:** Begin with a clear statement of the research objective. For example, "This study investigates the sulfur content in biodiesel produced from Calophyllum inophyllum L. seeds collected from various regions in Southern Karnataka."
- **Methods Brief:** Specify the primary analytical techniques used. For example, "Sulfur content was analysed using Inductively Coupled Plasma Optical Emission Spectroscopy (ICP-OES) and turbidimetry."
- **Quantitative Results:** Include specific quantitative results. For example, "The sulfur content in biodiesel ranged from X ppm to Y ppm across the studied regions."
- **Conclusions:** Conclude concisely about the findings and their implications, avoiding broad statements like "further research is needed." Instead, use, "The study highlights regional differences in sulfur content, which could impact biodiesel quality standards."

2. Introduction

Summary: The introduction contextualises the study within India's broader energy demand and fossil fuel dependency issues. It introduces Calophyllum inophyllum L.

as a potential feedstock for biodiesel and discusses the relevance of sulfur content analysis.

Strengths:

- Well-structured background information on the energy landscape and the significance of biodiesel.
- The justification for choosing *Calophyllum inophyllum* L. is based on its advantages and potential as a biodiesel source.

Areas for Improvement:

- The introduction could include a more precise statement of the study's objectives and research questions.
- More recent references could be added to support claims about the benefits of *Calophyllum* biodiesel.
- A brief overview of previous research on sulfur content in biodiesel would provide a better context.

Specific improvements to be done to the manuscript:

- **Clear Research Gap:** Clearly identify the gap in the existing literature. For instance, "While biodiesel from *Calophyllum inophyllum* L. has been explored for its high oil content, the impact of regional soil sulfur content on the sulfur levels in biodiesel remains underexplored."
- **Recent References:** Update the introduction with the most recent studies on biodiesel production, sulfur content, and non-edible feedstocks.
- **Objectives and Hypotheses:** Clearly state the study's objectives and any hypotheses. For example, "This study aims to evaluate the sulfur content in *Calophyllum* biodiesel and determine the correlation between soil sulfur content and the sulfur levels in the final product."

3. Materials and Methods

Summary: This section describes the study area, the processes used for oil extraction and biodiesel production, and the methods for analysing sulfur content.

Strengths:

- Detailed description of the study area, including geographic and climatic information.

- A comprehensive explanation of the methodologies used, including preparing sodium methoxide and the biodiesel washing process.

Areas for Improvement:

- Some methodological details are too specific and could be streamlined to focus on the key steps.
- The statistical methods used for data analysis should be clearly outlined.
- The section could benefit from a flowchart or diagram illustrating the process steps.

Specific improvements to be done to the manuscript:

- **Study Area Description:** While geographic and climatic details are included, consider summarising them and focusing more on their relevance to the study (e.g., variations in sulfur content in soil).
- **Methodological Clarity:** Simplify the methodology by highlighting critical steps. For example, "Oil extraction was performed using the Soxhlet method, followed by transesterification using sodium methoxide."
- **Statistical Analysis:** Clearly outline the statistical methods used for analysing the data, such as correlation analysis, regression models, or ANOVA, to show how data reliability and significance were determined.
- **Visual Aids:** Consider adding a flowchart or diagram of the methodology to enhance reader comprehension.

4. Results and Discussion

Summary: The results section presents data on sulfur content in soil, oil, and biodiesel across different regions. The discussion interprets these findings in the context of regional differences and edaphic factors.

Strengths:

- The data is well-organised, and the tables are informative.
- The discussion logically interprets the results, linking them to environmental factors.

Areas for Improvement:

- The presentation of results could be enhanced with more visual aids, such as graphs or charts.

- There should be a more apparent distinction between the presentation of results and their discussion.
- The discussion could delve deeper into the implications of the findings for the biodiesel industry and environmental policy.

Specific improvements to be done to the manuscript:

- Results Presentation: Separate the results and discussion more clearly. First, straightforwardly present the findings, then discuss their implications and context.
- Visual Data Representation: Include graphs or charts to visually represent data trends, such as sulfur content variations across different regions.
- In-depth Discussion: Provide a deeper analysis of the results. For example, discuss why certain regions have higher sulfur content and how soil characteristics or regional environmental factors might contribute.
- Comparative Analysis: If available, compare your findings with existing literature on similar studies. This helps contextualise the results within the broader scientific discussion.

5. Conclusion

Summary: The conclusion reiterates the potential of Calophyllum as a biodiesel feedstock and the influence of sulfur content on biodiesel quality. It suggests the need for further research to refine purification processes.

Strengths:

- The conclusion effectively summarises the essential findings and their implications.

Areas for Improvement:

- The conclusion could be more focused, avoiding the repetition of earlier sections.
- Recommendations for future research should be more specific, possibly suggesting methodologies or areas of focus.

Specific improvements to be done to the manuscript:

- Concise Summary: Summarise the key findings in a few sentences. Avoid reiterating detailed results; focus on the study's main contributions.

- **Specific Recommendations:** Offer particular recommendations for future research, such as "Further studies should explore purification methods to reduce sulfur content in biodiesel to meet international standards."
- **Practical Implications:** Discuss the practical implications of your findings for biodiesel production and policy, e.g., "The regional variability in sulfur content suggests the need for localised quality control measures in biodiesel production."

6. Language and Style

- **Grammar and Tenses:** The manuscript generally uses correct grammar and tenses, but there are occasional inconsistencies and minor grammatical errors.
- **Sentence Structure:** Some sentences are overly complex or lengthy, affecting clarity. Simplifying these sentences would improve readability.
- **Suggestions for Improvement:**
 - Conduct a thorough proofreading to correct minor grammatical errors and inconsistencies.
 - Simplify complex sentences and ensure consistent use of tense throughout the manuscript.
 - Use more active voice constructions to enhance clarity and engagement.

Original Research Article

INFLUENCE OF EDAPHIC FACTORS ON SULFUR CONTENT IN *Calophyllum inophyllum* L. BIODIESEL

ABSTRACT

Aims: The experiment investigated the sulfur content in *Calophyllum* biodiesel ~~in relation to, particularly the sulfur content~~ in *Calophyllum* kernel oil and in corresponding soil at different depths.

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Place and Duration of Study: The study area selected was Southern Karnataka region viz., Bengaluru (Plateau), Hassan (Plain) and Udupi (Coast).

Methodology: *Calophyllum* kernels collected at ~~4~~ four different places in each district were subjected to the oil extraction process, and ~~biodiesel was produced by an acid-base catalysed transesterification process~~ an acid-base catalysed transesterification process produced biodiesel due to the high acid value in oil. ~~As a catalyst, NaOH was used and methanol served as the analytical solvent in NaOH was used as a catalyst, and methanol served as the analytical solvent for~~ the transesterification reaction. ~~During the process, a 1:6~~ oil to methanol ratio was used at 60 °C reaction temperature. The sulfur content in soil, oil and biodiesel was calculated using ICP-OES through ~~the~~ CaCl₂ extractant method (turbidimetry).

Results: The study revealed ~~that better quality of biodiesel~~ biodiesel quality was observed in the Udupi region, followed by Bengaluru and Hassan, as Udupi (coast) is native to this species, and rainfall ~~also~~ determines the growth performance. Also, biodiesel produced in these three regions met ASTM D6751 and BIS (ISO 15607) standards. The sulphur content in CIME was ~~found to be~~ 7 ppm, 46 ppm and 46 ppm in Bengaluru, Hassan, and Udupi regions, respectively. The soil samples reveal that the total sulfur in the study area ranged from 10.31 ppm to 53.47 ppm. The samples collected from the Udupi region have shown higher sulfur content (42.49 to 53.47 ppm). The higher concentration of sulfur in biodiesel is due to the influence of edaphic factors.

Conclusion: Overall, ~~Calophyllum is found to be a suitable feedstock for biodiesel production by developing the methods for the purification process of the elements hindering the~~ *calophyllum* is found to be a suitable feedstock for biodiesel production by developing methods for purifying the elements that hinder its quality. These predictors, however, need further work to validate reliability.

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Keywords: *Calophyllum inophyllum* L.; Transesterification; NaOH (catalyst); biodiesel; Sulphur analysis

1. INTRODUCTION

India will overtake the European Union as the world's third-largest energy consumer by 2030, the International Energy Agency (IEA) said as it forecast India ~~accounting to account~~ for the ~~biggest-most significant~~ share of energy demand growth over the next two decades. This might be due to the country's dynamic economic ~~growthdevelopment~~, population growth, and modernisation over the past several years. Fossil fuels are pivotal in ~~the development and management of the global economy and are considered asdeveloping and managing the global economy and are~~ integral to a country's economic development. ~~When considering a developing country like India, itA developing country like India's~~ economy depends heavily on imports of fossil fuels from other countries.

The escalating petrol and diesel prices ~~and the declining reserves of fossil fuel along with growing environmental concerns are considered to be the major impetus for many initiatives to search for alternative sources of energy, declining reserves of fossil fuel, and growing environmental concerns are the primary impetus for many initiatives to search for alternative energy sources to,~~ which can supplement or replace fossil fuels. Biodiesel is one of the promising alternative energy sources for transport and mechanised agriculture sectors. Biodiesel is a non-toxic, biodegradable fatty acid methyl ester produced from edible ~~oils, non-edible oils, non-edible,~~ and animal fat.

Biodiesel can make a ~~major-significant~~ contribution in the future if it meets the few percent of petroleum and ~~it can provide improved fuel properties lower emission of unburned hydrocarbons, carbon monoxide but higher level of oxides of nitrogencan provide improved fuel properties, lower emission of unburned hydrocarbons and carbon monoxide and higher levels of nitrogen oxides~~ (Ali et al., 2013).

Biodiesel, as defined by the World Customs Organization (WCO), is "a mixture of mono-alkyl esters of long-chain [C 16-18] fatty acids derived from vegetable oils or animal fats which is a domestic renewable fuel for diesel engines and meets the international specifications (ASTM D 6751)." The transesterification process produces esters from vegetable oil. It is a ~~process of reactionreaction process~~ between triglycerides and alcohol in the presence of a catalyst to produce glycerol and ester (biodiesel).

The Government of India has formulated the National Policy on Biofuels ~~which was introduced in the year 2008, was, which was introduced in 2008 and~~ approved by the Union Cabinet in May 2018. The policy aimed ~~at takingto take~~ forward the indicative target of achieving 20% blending of biofuels with fossil-based fuels by 2030. The government has set some ambitious goals for the energy sector, which include electrification of all census villages by 2019, 24x7 electricity and 450 GW of renewable energy capacity by 2030, reduction in energy emissions intensity by 33%-35% by 2030, and production above 40% electricity from non-fossil fuels by 2030. These goals exhibit the Centre's push towards strengthening the ~~energy infrastructure of the country while promoting the agenda ofcountry's energy infrastructure while promoting~~ sustainability. To achieve this target ~~more and more feedstocks are to be explored, more and more feedstocks are to be explored, along~~ with technology for handling these feedstocks for biodiesel production.

There are different potential feedstocks for biodiesel production. The use of edible vegetable oils or ~~the first-generation feedstock has been of great concern recently; this-is~~ because they raise many concerns ~~about~~ the issue of ~~the~~ food versus fuel debate. Therefore, non-edible vegetable oils or ~~the second-generation feedstocks have become more attractive for biodiesel production. A substantial number of non-edible oilseed plants were being identified which have the potential to, which could~~ be used as biodiesel feedstocks. Pongamia has

been successfully proven as a potential ~~tree-tree-born oils for biodiesel production in India for India's biodiesel production~~. A few more oils are to be explored to meet the ~~huge-massive~~ demand for biodiesel. ~~One~~ such oil could be Calophyllum oil (surahonne oil), ~~which is~~ obtained from the kernels of Calophyllum inophyllum L. fruit. It is locally with ~~common~~ ~~familiar~~ names— Alexandrian laurel (English), Punnai (Tamil), Surahonne (Kannada), and Undi (Marathi – Maharashtra), ~~etc.~~

Calophyllum inophyllum L., commonly known as Polanga, is an inedible oilseed, ornamental evergreen tree belonging to the Clusiaceae family ~~that is found~~ in tropical regions of India, Malaysia, Indonesia, and the Philippines. Typically growing up to 25 m in height, the Polanga tree produces a slightly toxic fruit that contains a single, large seed. The oil obtained from polanga seeds is high in FFA content (up to 22 wt. %) and unsaturated species such as linoleic (38.3 wt. %) and oleic (34.1 wt. %) acids. ~~The~~ remaining fatty acids found in polanga oil are stearic (13.0 wt. %) and palmitic (12.0 wt. %) acids, with a trace amount of linoleic acid (0.3 wt. %/0) (Sahoo et al., 2007).

1.1. Botanical description of *Calophyllum inophyllum* L.

Calophyllum inophyllum L. belongs to the plant family Clusiaceae (Mangosteen family). The plant is named for its beautiful leathery leaves. *Calophyllum* grows in mixed cultures with minimal cultivation, ~~also and~~ in previously cleared and degraded lands. The tree naturally grows in the sub-tropical and tropical atmosphere (~~within the temperature~~ between 18 and 33 °C) and free-draining soils close to shorelines. It is frequently found in clay soils within Australia, India, Sri Lanka, and ~~throughout~~ central and southern Asia, including Indonesia. In India, ~~the states like Maharashtra, Gujarat, Madhya Pradesh, Andhra Pradesh, Karnataka, and Tamil Nadu are blessed with a considerable amount~~ ~~states like Maharashtra, Gujarat, Madhya Pradesh, Andhra Pradesh, Karnataka, and Tamil Nadu are blessed with many of~~ *Calophyllum* trees.

The tree is a low-branching and slow-growing tree with two distinct flowering periods ~~of~~: late spring and late autumn. ~~In most parts of the world, the tree shows two flowering and fruiting seasons~~ ~~The tree shows two flowering and fruiting seasons in most of the world~~. However, sometimes flowering may occur throughout the year. The tree supports a dense canopy of glossy, elliptical, shiny, ~~and~~ tough leaves, fragrant white flowers, and large round drupes. Its size typically ranges between 8–20m (25–65ft) tall at maturity, sometimes reaching up to 35m (115ft). The ~~growth rate of the tree's~~ ~~growth rate~~ is 1m (3.3ft) in height per year on ~~good-suitable~~ sites. Its leaves are heavy and glossy, 10– 20cm (4–8in.) long and 6–9cm (2.4–3.6in.) wide, light green when young and dark green when older. Fruits are spherical drupes and arranged in clusters. The fruit is pinkish-green, later turning bright green, and when ripe, it turns dark grey-brown and wrinkled. The tree yields 100–200 fruits/kg. In each fruit, one large brown seed 2–4cm (0.8–1.6in.) in diameter is found. The single, large seed is surrounded by a shell (endocarp) and a thin, 3–5mm ~~layer of pulp~~ ~~pulp layer~~. The oil is tinted green, thick, and ~~has a~~ woody or nutty smell. Oil yield per unit of land area has been reported at 2000kg/ha. According to Atabani and Silva (2014), the kernels have higher oil content in the ~~range of 43%–75%~~ ~~43% - 75% range~~. *Calophyllum* was recognised as one of the most potential feedstock for biodiesel production as a result of the high oil productivity of the seeds.

1.2. Advantages of *Calophyllum inophyllum* L.

There are many advantages of using *Calophyllum inophyllum* L., such as high survival potency in nature, ~~productive up to 50 years~~ ~~up to 50 years of productivity~~, and higher oil yield. These trees serve as ~~a windbreaker at the seashore where it can reduce abrasion,~~

~~protect crops, provide ecotourism, and conservation of windbreakers at the seashore, where they can reduce abrasion, protect crops, provide ecotourism, and conserve~~ coastal demarcation. Its biodiesel meets the US ASTM D 6751 and European Union EN 14214 biodiesel standards. Calophyllum biodiesel can be used as a potential substitute for diesel and possesses better lubrication capability.

1.3. Need of the study

However, the potential of Calophyllum oil as a source of second-generation biodiesel is yet to be utilised commercially because of the absence of knowledge on the production process and biodiesel quality. Calophyllum biodiesel properties like viscosity (6.0 cSt at 40 °C), density (0.88 kg/m³), calorific value (34 MJ/kg), flash point (178 °C), and fire point (196 °C) all were within the specified limit (Madhusudana, 2010).

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Biodiesel quality control also involves the determination of sulfur content. It plays a ~~very important~~^{crucial} role because it modifies the efficiency of biodiesel production ~~as well as~~ the stability of these products. Furthermore, they are toxic and generate environmental concerns, whereas others are used as additives. ~~Normally Biodiesel and bioethanol are typically, products such as biodiesel and bioethanol are~~ mixed with conventional fossil fuels ~~such as~~ (diesel and gasoline, ~~respectively~~). Therefore, elements come from the raw product employed for biofuel production (seeds) ~~as well as from the production and storage process or even from, the production and storage process, or even~~ the added fuels. The elements in raw materials may come from edaphic factors.

— In the case of Calophyllum, ~~the presence of~~ some elements may deteriorate ~~the quality of biodiesel~~^{biodiesel quality}. Hence, the influence of edaphic factors on the quality of Calophyllum biodiesel ~~are is~~ needed to be studied. With this background, the present study is undertaken to analyse the suitability of Calophyllum oil for ~~the production of biofuel~~^{biofuel production} and to evaluate the quality of the biodiesel produced.

2. MATERIAL AND METHODS

2.1. Study area

The present study was ~~carried out in the southern districts of Karnataka, viz~~^{conducted in the southern districts of Karnataka}. Bangalore, Hassan, and Udupi. Bangalore and Hassan are located in the southern interior region of Karnataka, while Udupi is in the southern coastal region.

Bangalore (Bangalore Urban) is ~~the capital city of Karnataka, covers Karnataka's capital city,~~^{covering} an area of about 2,196 sq. km. It lies between 12.9716° North latitude and 77.5946° East longitude at a mean altitude of 920 m above mean sea level and receives an average annual rainfall of 920 mm.

Hassan district lies between ~~13.0753~~^{13.0753} ° N latitude and 76.1784° E longitude, ~~with an extent of about of~~^{totaling} 6826.15 Sq. km at ~~an altitude of~~^{980 m (mean)} above mean sea level and receives an average annual rainfall of 806 mm. The geography is mixed with the Malnad or mountainous region to the west and southwest ~~called Bisle Ghat, Bisle Ghat,~~ and the ~~maidan~~^{Maidan} or ~~plains~~^{Plains} regions in the north, south, and east.

Udupi district covers an area of about 3,582 sq. km. It lies between 13.3409° North latitudes and 74.7421° East longitude ~~at an altitude of~~ s at 9 m above mean sea level and receives an average rainfall of 4360 mm annually.

2.2. Determination of kernel oil content

The estimation of kernel oil content was done by using the Soxtherm apparatus. This works on the principle of the solvent extraction process.

2.2.1. Procedure

The dried seeds of *Calophyllum inophyllum* L. were powdered in a mixer grinder. Then, 4 g of powdered samples were weighed. Then, the samples were placed inside the cotton thimble and plugged with cotton. Then, the weight of the Soxtherm jars containing boiling stones was taken, and a cotton thimble was placed inside the jar with the help of a hanger. 100 ml of petroleum ether was added to each jar and placed into the Soxtherm apparatus. The oil extraction was performed by running the pre-programmed Soxtherm apparatus for 3 hours 27 minutes. To remove the remaining petroleum ether and moisture, the extracted oil was subjected to oven drying for 1 hour at 110 °C after the completion of the oil extraction process. The jars ~~are then placed inside the desiccator containing CaCO₃ for one hour~~ were then placed inside the desiccator containing CaCO₃ for one hour, and the final weight of the jar containing oil was noted. The following formula was used to calculate the kernel oil content.

$$\text{Kernel oil content (\%)} = ((W_2 - W_1)/W) \times 100$$

Where,

W = ~~Weight-weight~~ of powdered kernel sample

W₁ = Weight of Soxtherm jar along with boiling stones

W₂ = Weight of Soxtherm jar with boiling stones and extracted oil

2.3. Biodiesel Production from Calophyllum oil

2.3.1. Biodiesel production

Biodiesel was produced by pooling the oil samples collected from Bangalore, Hassan, and Udupi districts separately and analysed for its nature and properties, yield, ~~quality-as well as,~~ and the potentiality of biodiesel. The ~~quality-of biodiesel~~ biodiesel quality obtained from the kernel was within the standards prescribed by ASTM. The biodiesel was produced from the kernel oil by transesterification using methanol and NaOH/KOH as a catalyst to yield fatty acid methyl ester (FAME ~~or~~ which is known as biodiesel) and glycerine as a by-product. Similar findings were also reported by Ma et al. (1998).

2.3.2. Pre-treatment process

Oil (a known amount, i.e. 500 ml) was taken in ~~one-a one-liter-litre~~ three-necked flask fitted with a reflux condenser, catalyst dozer, and temperature sensor. The oil was heated to ~~a-the~~ required temperature (55-60 °C) on a magnetic stirrer with a temperature controller.

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2.3.3. Acid esterification process

The esterification process is used when the ~~free fatty acid (FFA) content of the refined oil~~refined oil's free fatty acid (FFA) content exceeds 2%. The FFA content of Calophyllum oil was in the range of 4.4% - 9.9%. Therefore, a two-step acid-base catalysed transesterification was adopted to convert crude *Calophyllum inophyllum* oil (CCIO) into *Calophyllum inophyllum* methyl ester (CIME). 100 ml methanol was added to 500 ml oil, and 2 ml Sulfuric acid was added and heated at 55 °C with a magnetic stirrer for 1 hr. On completion of this reaction, the products were poured into a separating funnel to separate the excess alcohol, sulfuric acid, and impurities presented in the upper layer. The lower layer was separated. This process was repeated using the same methodology to reduce the FFA content to 2%.

2.3.4. Trans-esterification process

2.3.4.1. Preparation of sodium methoxide

Sodium methoxide was prepared by dissolving NaOH in anhydrous methyl alcohol (> 99 %) in a conical flask.

2.3.4.2. Procedure

~~Sodium methoxide was added slowly to the esterified oil obtained from the previous step with constant stirring~~With constant stirring, sodium methoxide was added slowly to the esterified oil obtained from the previous step. The trans-esterification reaction was carried out for 2 hours at 60 °C. Then, it was subjected to settling in a separating funnel to form an upper biodiesel layer and a lower glycerine layer. The bottom glycerine layer was drained ~~out~~, and the biodiesel obtained in the upper layer was collected.

2.3.5. Biodiesel washing

Biodiesel thus produced was washed three to four times with an equal quantity of water acidified with acetic acid (0.1%) to avoid emulsification. Further, the biodiesel was washed twice with water to remove the soluble contaminants. Biodiesel was then dried by heating at 110 °C till the moisture content was ~~removed completely~~obliterated. It was cooled and filtered, then subjected to further analysis.

2.4. Digestion of Oil and Biodiesel

Approximately 0.4g of oil sample was weighed and transferred to the digestion tube, and 8 ml of concentrated nitric acid was added. Then, the tubes are placed in Gerhardt's digestion block for digestion until the appearance of a white precipitate. The same procedure was repeated for biodiesel digestion. Then, the digested samples were made to 25 ml using distilled water and utilised for further analysis.

2.5. Analysis of Sulfur concentration in soil, oil and biodiesel

~~The Sulfur content of the samples~~ was analysed by ICP-OES through ~~the~~ CaCl₂ extractant method (Turbidometry) (Black, 1965).

3. RESULTS AND DISCUSSION

3.1. Survey and Documentation of *Calophyllum inophyllum* L. along Southern Karnataka

Calophyllum inophyllum L. trees were distributed along the southern Karnataka region, especially in coastal areas and ~~also in~~ interior land. The survey ~~work was carried out as~~ conducted in three districts of Southern Karnataka viz., Bangalore, Hassan, and Udupi. Bangalore and Hassan are located in the southern interior region of Karnataka, while Udupi is in the southern coastal region.

Table 1: Different study sites of *Calophyllum inophyllum* L. in Bangalore, Hassan, and Udupi districts with latitude, longitude, and altitude.

S.No.	Districts	Locations	GPS points		
			Latitude	Longitude	Altitude(m)
1	Bangalore	Lalbagh 1	12° 57' 2.41" N	77° 35' 1.14" E	906.60
2		Lalbagh 2	12° 57' 1.8" N	77° 35' 0.96" E	887.40
3		GKVK - 1	13° 5' 27.85" N	77° 33' 56.48" E	1036.28
4		GKVK - 2	13° 4' 44.54" N	77° 34' 52.5" E	944.00
5	Hassan (Biofuel parkPark, Madenur)	Hassan 1	12° 58' 32.63" N	76° 16' 6.24" E	917.00
6		Hassan 2	12° 58' 33.6" N	76° 16' 6.89" E	906.20
7		Hassan 3	12° 58' 33.06" N	76° 16' 6.85" E	945.30
8		Hassan 4	12° 58' 33.06" N	76° 16' 6.42" E	924.90
9		Hassan 5	12° 58' 33.71" N	76° 16' 7.07" E	930.10
10	Udupi (UD)	UD - Kapsi, Kalthodu	13° 49' 39.94" N	74° 40' 28.99" E	90.76
11		UD- Gantihole	13° 49' 39.54" N	74° 40' 28.2" E	5.20
12		UD- Poduvari	13° 49' 51.21" N	74° 39' 37.3" E	5.60
13		UD- Dombi, Shiroor	13° 49' 51.21" N	74° 39' 37.3" E	5.60

3.2. Oil content of *Calophyllum* kernels

The oil content of *Calophyllum* seed kernels was determined by using Soxtherm extraction. The results obtained in this study are presented in ~~table-Table~~ 4.19. There is a significant variation in the oil content of the kernels collected from different locations. The content of oil ranged from 52.67% to 73.95%. The maximum oil content was noticed in the kernels from Dombi, Udupi (73.95 %), and the ~~least oil content was noticed~~ lowest was in GKVK 2 (52.67 %). The oil content of *Calophyllum* in Bangalore, Hassan, and Udupi varied from 52.67 to 68.36 percent, 56.96 to 59.94 percent, and 62.08 to 73.95 percent, respectively. Udupi district samples recorded more oil content ~~compared to than~~ other districts. This might be due

to higher nitrogen content in Udipi soil, which influenced the oil content. Similar results were reported by Shilpi *et al.*, (2014) in sesame oil content. It is indicated in Fig. 1.

~~The formation of oil~~Oil formation is a complex biochemical pathway in which the enzymes play a major significant role. Environmental conditions such as humidity, temperature, and soil characteristics are important parameters that influence the oil content in seeds. The percentage of oil in the kernels of Calophyllum was found to be higher compared to the other oil-yielding species. The oil percentage ranged from 47% to 75%, as reported by Atabani and Silva (2014)Atabani and Silva (2014) reported. It is higher than Simaruba, Mahua, Jatropha, Pongamia, and Neem. Similar findings were reported by Pant *et al.*, (2006), where they (2006), who found that Jatropha oil content varied depending on the type of species and climatic conditions but mainly on the altitude where it is grown. Manian and Gopalakrishnan, (1995), also reported similar findings that there was a higher utilisation of photo-assimilation for plant growth, compared to (1995) also reported similar findings that photo-assimilation was utilised more for plant growth than oil production at higher altitudes.

Table 2:Oil content in Calophyllum kernels

S. No.	Location	Oil content (%)
1	Lalbagh 1	62.28 ⁱ
2	Lalbagh 2	68.36 ^j
3	GKVK – 1	59.34 ^e
4	GKVK – 2	52.67 ^a
5	Hassan 1	59.94 ^g
6	Hassan 2	56.96 ^b
7	Hassan 3	58.37 ^d
8	Hassan 4	59.60 ^f
9	Hassan 5	57.03 ^c
10	UD - Kapsi, Kalthodu	62.08 ^h
11	UD – Gantihole	72.58 ^l
12	UD – Poduvari	70.85 ^k
13	UD – Dombi, Shiroor	73.95 ^m
	Mean	0.05
	SE(m)	0.02
	C.D.D (5%)	0.02

	CV (%)	0.04
Note: Superscript alphabets (a to m) indicate significance at the 0.05 level		

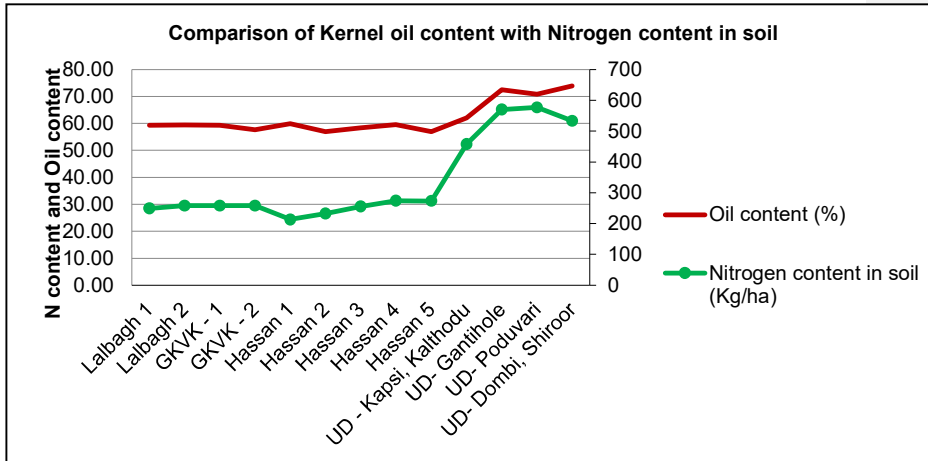


Fig. 1: Comparison of Kernel oil content with Nitrogen content in Soil

3.3. Determination of Sulfur content in Soil and Oil:

The soil samples collected under each tree in 13 different locations at the depth of 0-15 cm and 15-30 cm were subjected to the analysis of sulfur content. Total sulfur in the study area ranged from 10.31 ppm to 53.47 ppm. The samples collected from the Udupi region have shown higher sulfur content (42.49 to 53.47 ppm). The sulfur content is in accordance with the pH of the soil, which is in the acidic range. This might be due to the sulfur which is converted to sulfuric acid and gives an acidic range of pH, which is converted to sulfuric acid and gives an acidic pH range in that region. Similar results were obtained by Motowicka and Terelak (1998). Sulphur is available to plants in the form of sulphates. Sulphates dissolved in the water column is are mostly assimilated assimilated mainly by plants and incorporated into amino acids, i.e. cysteine and methionine (Gao *et al.* 2000, Sievert *et al.* 2007). However, some are also bound to sulphated polysaccharides in the oxidised form.

The S content is found to be higher in the Udupi region (72 to 88 ppm) compared to Hassan (42 to 67 ppm) and Bangalore (52 to 59 ppm). This might be due to its occurrence in the soil at a higher rate.

Table 3: Sulphur(S) content in soil in at different depths and in oil at different various locations

Location	Sulphur (ppm) in soil	S(ppm)
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	Soil Depth			in Oil
	0-15 cm	15-30 cm	Mean	
Lalbagh 1	10.8	9.83	10.31 ^a	58
Lalbagh 2	18.24	16.89	17.56 ⁱ	59
GKVK – 1	12.84	12.17	12.51 ^f	56
GKVK – 2	12.51	12.14	12.32 ^e	52
Hassan 1	16.16	11.77	13.97 ^g	57
Hassan 2	16.16	13.25	14.70 ^h	42
Hassan 3	12.49	11.26	11.88 ^d	48
Hassan 4	11.73	9.63	10.68 ^c	67
Hassan 5	11.28	9.58	10.43 ^b	67
UD – Kapsi, Kalthodu	42.02	42.97	42.49 ^j	72
UD – Gantihole	44.88	43.97	44.42 ^k	87
UD – Poduvari	49.66	51.55	50.60 ^l	82
UD – Dombie, Shirur	52.51	54.42	53.47 ^m	88
Mean	23.94 ^b	23.03 ^a		
	Location	Depth	Interaction	
SE(m)	0.012	0.005	0.016	6
C.D.D (5%)	0.034	0.013	0.048	13
CV (%)	0.135			6.88
Note: Superscript alphabets (a to m) indicate significance at the 0.05 level				

3.4. Biochemical characteristics of biodiesel

The quality of biodiesel is expressed in terms of the ~~fuel properties such as acid value, iodine value, saponification value, density, viscosity, calorific value and sulfur content of the Calophyllum biodiesel~~ Calophyllum biodiesel's fuel properties, such as acid, iodine, saponification, density, viscosity, calorific, and sulfur content. All are well within the ASTM

and BIS standards. Similar results were reported by Madhusudana (2010). The data obtained from the study are given in the table

Table 4: Bio-chemical Characterisation of Biodiesel

Biodiesel Parameters	ASTM D6751	BIS (ISO 15607)	Diesel	Biodiesel			C.D. D (At 5%)	SE(m)	C.V. (%)
				Bangalore	Hassan	Udupi			
Acid value (mgKOH/g)	Max 0.80	0.5 Max	0.02	0.232 ^b	0.4 ^c	0.072 ^a	0.07	0.02	5.23
Iodine value (gI ₂ /100g)	-	120 Max	-	66.48 ^c	47.19 ^a	57.49 ^b	1.18	0.38	1.48
saponification value (mgKOH/g)	-	-	-	185.49 ^a	202.97 ^b	184.90 ^a	13.00	4.17	4.40
Calorific value (MJ/kg)	-	-	44.96	39.86 ^c	39.13 ^b	38.15 ^a	0.03	0.01	0.05
Density (kg/m ³) at 40°C	860-900	860-900	840	874.18 ^c	891.14 ^b	873.82 ^a	1.96	0.629	0.16
Viscosity (cSt)	1.9 - 6.0	2.5-6.0	3.12	5.56 ^a	5.98 ^c	5.78 ^b	0.06	0.02	0.67
Cloud point Temp.(°C)	Max 18	-	-	9.8 ^a	15.0 ^c	11.4 ^b	0.08	0.03	0.46
Pour point Temp.(°C)	-	-	-	6.0 ^a	9.0 ^b	6.0 ^a	0.08	0.02	0.78
Flashpoint (°C)	Min 130	-	55	168.0 ^c	158.0 ^a	166.0 ^b	0.76	0.25	0.33
Sulphur (ppm)	Max 500	-	-	7 ^a	46 ^b	46 ^b	1	0	1.489
FAME content (%)	-	96.5 Min	-	90.6 ^b	73.5 ^a	99.7 ^c	0.096	0.031	0.078

Note: Superscript alphabets (a to c) indicate significance at the 0.05 level

3.5. Sulphur content in Calophyllum biodiesel

Sulphur emissions, both gaseous and particles, are harmful to human health. Acute exposure can cause trouble breathing, and long-time exposure to those emissions can cause heart disease, pulmonary illness, or even untimely death. In the environment, sulfur oxides are reactive and form H₂SO₄, which comes down with the rain, and the acid rain again depletes nature in many ways. Moreover, buildings disintegrate because of acid rain. (Pan, 2011). Sulfur compounds in biodiesel are present in different forms, such as hydrogen sulfide, sulfides, sulfur dioxide, mercaptans, and thiophenes.

The sulfur content in biodiesel should be within 500 ppm (ASTM D6751). Sulphur content in Calophyllum biodiesel varied from 7 ppm to 46 ppm. The concentration is within the prescribed limits.

Amongst, ~~the higher concentration is found in Hassan and Udupi and the least these, the~~ highest concentration is in Hassan and Udupi, and the lowest is in Bangalore. This might

due to the presence of a higher concentration of sulfur in the soils of Hassan and Udupi. The simple relationship of sulfur content in which each district sample's sulfur content was taken as average in the case of soil (irrespective of depth) and oil, then compared with sulfur content in biodiesel. The relationship between the sulfur content in soil, oil and biodiesel were presented in Fig.2. This shows that edaphic factors might have influenced the sulfur content in biodiesel.

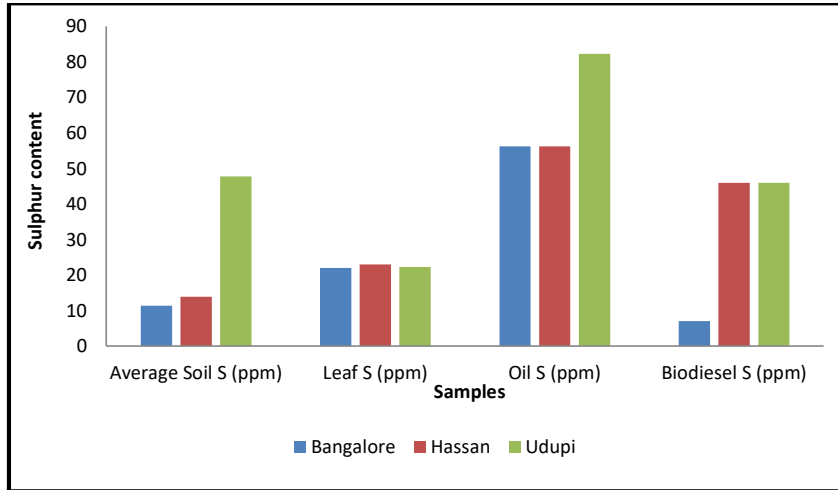


Fig.2: Sulphur content in soil, oil and biodiesel relationship

4. CONCLUSION

The present study entitled "Influence of edaphic factors on Sulfur content in *Calophyllum inophyllum* L. Biodiesel" was conducted to study the potentiality of *Calophyllum* biodiesel.

The study conducted on *Calophyllum* kernel oil content recorded that Udupi samples contain more oil content than other districts. This might be due to higher nitrogen content in Udupi soil, which influenced the oil content. The oil available sulfur content in the soil has shown on par relationship. This indicates that most of the elements in oil might be due to its presence in soil.

Due to the high acid value (FFA>4%) of *Calophyllum* oil in all the locations, it has to pass through a two-stage process during biodiesel production. The transesterification process was carried out using NaOH and methanol as catalysts. A 1: 6 oil to methanol ratio was used at 60°C reaction temperature. The maximum biodiesel conversion under optimum condition was observed in Udupi samples (99.7%) and the least in Hassan samples (73.5%). The properties of biodiesel, such as acid, iodine, saponification, calorific, viscosity, density, and FAME content, were also studied. There was a drastic decrease in acid value, density, and viscosity of biodiesel compared to oil. This might be due to the transesterification process. The calorific value increased.

The highest concentration of sulfur was found in Hassan and Udupi, and the lowest in Bangalore. This might be due to a higher concentration of sulfur in the soils of Hassan and Udupi. This shows that edaphic factors might have influenced the sulfur content in biodiesel. All three biodiesels produced, one from each district, were well within the prescribed ASTM standards.

These elements should be removed for usage as biodiesel. The purification process with conventional methods, such as wet washing with water or acidified water, was not found to remove these impurities. The other techniques, likely Vacuum distillation, had to be performed.

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UNDER PEER REVIEW



Latitude: 13.827653
Longitude: 74.674503
Altitude: 5.2 m
Accuracy: 3.4 m
Time: 10-03-2020 10:23

Gantihole - Udupi



Latitude: 13.830893
Longitude: 74.660355
Altitude: 5.6 m
Accuracy: 13.7 m
Time: 10-03-2020 11:47

Dombi - Udupi



Latitude: 13.827782
Longitude: 74.674767
Elevation: 92.66 m
Accuracy: 3.2 m
Time: 10-03-2020 09:45

Kapsi - Udupi



Latitude: 12.950248
Longitude: 77.583665
Elevation: 981.0m
Accuracy: 1.3m
Time: 11-19-2019 14:28

Lalbagh 2 - Bangalore

Plate 1: *Calophyllum inophyllum* L trees selected for study in different locations



GKVK 1 - Bangalore



Hassan 1

Plate 2: *Calophyllum inophyllum* L trees selected for study in different locations



Fibrous leaves



Fragrant White Flowers



Fresh fruits in tree



Dried fruits



Dried kernels



Oil and Biodiesel

Plate 3: *Calophyllum inophyllum* L leaves, flowers, fresh and dried fruits, dried kernels, kernel oil and biodiesel



Plate 4: Apparatus used for transesterification process



Biodiesel settling



Biodiesel washing

Plate 5: Apparatus used in the biodiesel production process for Biodiesel settling and washing