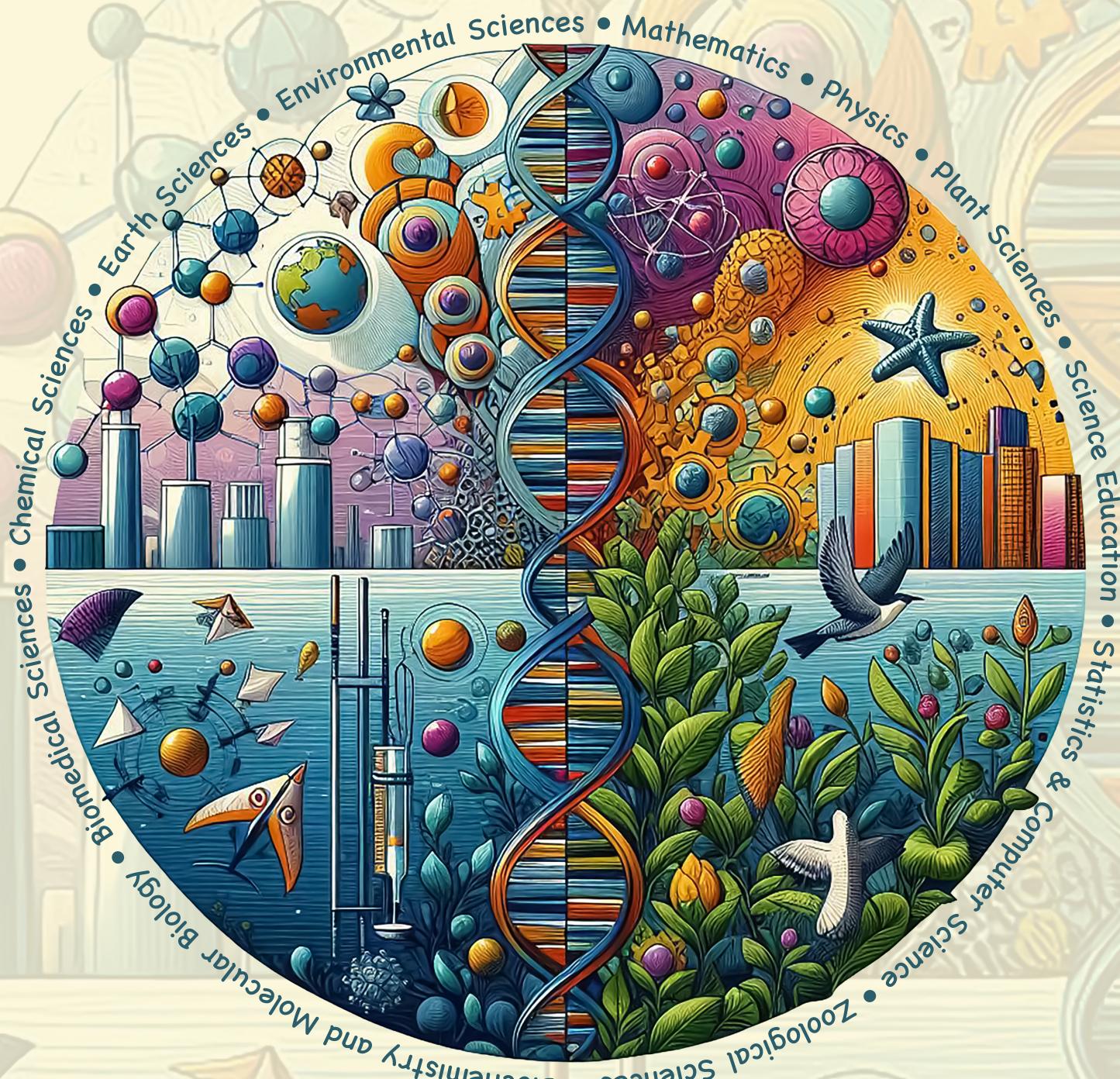




POSTGRADUATE RESEARCH HIGHLIGHTS 2024

Postgraduate Institute of Science University of Peradeniya, Sri Lanka



**POSTGRADUATE INSTITUTE OF SCIENCE
UNIVERSITY OF PERADENIYA
SRI LANKA**



**POSTGRADUATE
RESEARCH HIGHLIGHTS 2024
1st and 2nd November 2024**

RESCON 2024

PGIS Research Highlights 2024

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**Message from the Director, Postgraduate institute of Science,
University of Peradeniya**

The Postgraduate Institute of Science (PGIS) is committed to fostering a culture of excellence and innovation, offering a broad spectrum of postgraduate programmes, including diplomas, Master, M.Sc., M.Phil., and Ph.D. degrees. Our students engage in cutting-edge research across well-equipped laboratories at PGIS, the University of Peradeniya, and prominent national and international research institutions. Recently, PGIS has also introduced a research grant programme to further support and enhance our students' academic pursuits.

The research conducted by our postgraduate students plays a crucial role in advancing knowledge, innovation, and societal progress. By fostering critical thinking and scientific inquiry, postgraduate research lays the foundation for future discoveries, drives technological advancements, and enhances the skills of emerging scientists and experts. This ongoing pursuit of knowledge not only enriches academia but also fuels industries and addresses global challenges in scientific disciplines, sustainability, and more, making research indispensable for a resilient and progressive society.

The Research Highlights publication, released in conjunction with the PGIS Research Congress 2024, is an integral part of our mission to promote the visibility and impact of our researchers. This initiative helps young scientists establish their academic profiles and gain recognition for their work, which is crucial for their professional development. It is essential that the contributions of junior researchers are properly acknowledged, as their work forms the foundation for future scientific advancements.

I extend my sincere appreciation to Dr. Rajitha Ranasinghe, the Editor-in-Chief, along with his support team, for their dedicated efforts in bringing this publication to life. I also express my gratitude to all the M.Sc. (SLQF 10), M.Phil., and Ph.D. graduates and their supervisors for providing their research summaries and consenting to their publication in this volume. Their work continues to inspire and elevate the standard of research at PGIS.

Prof. H.M.T.G.A. Pitawala

Director
Postgraduate Institute of Science,
University of Peradeniya, Sri Lanka

Message from the Chairperson RESCON 2024

I am honored to share my message in this year's volume of Research Highlights as the Chairperson of RESCON 2024. This publication celebrates the dedicated research efforts of our M.Sc. (SLQF 10), M.Phil., and Ph.D. students at the Postgraduate Institute of Science, showcasing their impactful work and contributions across diverse scientific fields. Their research not only advances our understanding of critical issues but also enriches the knowledge base that drives our collective scientific progress.

The theme for RESCON 2024, *Breaking Boundaries: Collaborative Science for Global Challenges*, aligns seamlessly with the objectives of this Research Highlights volume. By publishing these research highlights along with the authors' profiles, we provide a platform for the broader scientific community to access the achievements of emerging scientists at the PGIS. At the same time, it is a stepping stone for the authors in their scientific careers ahead of publication in high-profile journals.

I extend my heartfelt thanks to the Director of PGIS, Prof. H.M.T.G.A. Pitawala, for his unwavering support of this publication and to Dr. Rajitha Ranasinghe, whose meticulous efforts have brought this volume to life. I am deeply grateful to each supervisor and author who has contributed to this compilation, and to the subcommittee members who have ensured the quality of each featured piece.

As you explore the outstanding research presented within these pages, I encourage you to connect with the authors and contribute to the collaborative spirit that both RESCON 2024 and this publication embody. This volume is a testament to the commitment of our postgraduate students and their mentors, and I wish all contributors every success with this 9th volume of the *PGIS Research Highlights 2024*.

Prof. Priyanga Wijesinghe

Department of Botany,
Faculty of Science,
University of Peradeniya, Sri Lanka

Message from the Editor-in-Chief

It is with great pride and enthusiasm that I present this message upon the successful completion of the PGIS Research Highlights - 2024. This marks the ninth volume in a series that began in 2016, showcasing the outstanding research contributions from our postgraduate community.

Postgraduate research—whether through Ph.D., M.Phil., or M.Sc. programmes—empowers scholars to deeply explore their fields, fostering innovation and critical thinking. These advanced degrees not only enhance individual expertise but also contribute significantly to the global body of knowledge, addressing complex societal challenges. At the Postgraduate Institute of Science (PGIS), University of Peradeniya, we are committed to cultivating an environment where passionate researchers can thrive, collaborate, and make impactful discoveries.

This volume encapsulates the remarkable achievements of seven Ph.D., fourteen M.Phil., and nine M.Sc. (SLQF 10) graduates, representing a significant portion of the forty-five research degrees awarded in 2023. I extend my heartfelt congratulations to all the postgraduates who have earned their degrees, as well as to their supervisors, whose invaluable guidance has facilitated the dissemination of these significant findings to the scientific community. This document will be available online on the RESCON 2024 website, ensuring worldwide access and paving the way for collaborative research while inspiring emerging scholars.

I would like to express my sincere appreciation for the untiring dedication and commitment of my editorial team throughout the preparation of this document; without their contributions, this achievement would not have been possible. Last but not least, I extend my gratitude to Prof. H.M.T.G.A. Pitawala, Director of PGIS, and Prof. Priyanga Wijesinghe, Chairperson of RESCON 2024, for their support and trust in allowing me to prepare this document that highlights the exceptional research output from PGIS in 2023.

Dr. Rajitha Ranasinghe

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BIOCHEMISTRY AND MOLECULAR BIOLOGY

NUTRITIONAL AND BIOCHEMICAL PROPERTIES OF RAW AND PROCESSES *Artocarpus nobilis* SEEDS

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I. INTRODUCTION

Nuts and seeds are recommended for a healthy diet, being recognized as a source of high-quality protein, lipids, and bioactive compounds (Kris-Etherton et al., 2008). Numerous epidemiological and clinical studies have revealed that consumption of nut and seeds is associated with the reduction of cardiovascular disease, coronary heart disease, hypertension, inflammation, type two diabetes, and cancer (De Souza et al., 2017). Consequently, several nut-based commercial industries have been developed to improve the consumers' health. Although, there are many indigenous species of edible nuts and seeds in developing countries that have great potential to address food insecurity, insufficient research has been conducted to explore their possibilities.

Artocarpus nobilis, commonly known as Ceylon breadfruit is a native plant in Sri Lanka that produces seeds with a unique taste. Belonging to the family Moraceae, *A. nobilis* is reported to be the only endemic species in Sri

Lanka, mainly distributed in the Southern wet zone of the country (Jayasinghe et al., 2004). Despite its potential, the seeds of *A. nobilis* remain underutilized and unexplored for their nutritional and functional properties.

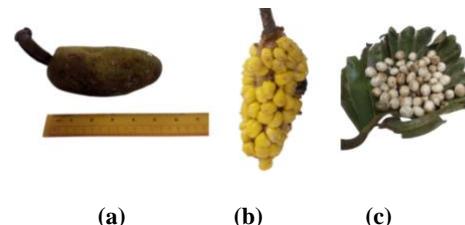


Fig. 1: *Artocarpus nobilis* (a) mature fruits (b) Flesh (c) Seeds

The objectives of the current study were,

- i. Determination of nutritional properties of raw and processed *Artocarpus nobilis* seeds
- ii. Proximate composition, mineral content, dietary fiber content, starch composition
- iii. Evaluation of biochemical properties of raw and processed *A. nobilis* seed
- iv. Phytochemical analysis, phenolic profile, anti-oxidant activity, α - amylase inhibitory activity, prebiotic activity, fatty acid composition
- v. Investigate the effect of 3% roasted *A. nobilis* seed powder incorporated experimental diets on feed intake, growth parameters, fasting lipid profile, and fasting glucose concentration of adult male Wistar rats

II. METHODOLOGY

Mature seeds of *A. nobilis* were collected from eight location in Sri Lanka. Seeds were authenticated by the National Herbarium of the Peradeniya Botanical Garden. A pool of raw seeds was divided into four groups and subjected to three different processing methods: pan-roasting, boiling, and microwaving.

A. Determination of nutritional properties

Moisture, lipid, crude protein, ash, dietary fiber, and carbohydrate contents were analyzed according to the relevant standard procedure described in the AOAC (AOAC 2012). The dietary fiber content of *A. nobilis* seed was analyzed using a dietary fiber assay kit (TDF 100A-1KT) purchased from Sigma-Aldrich. In the determination of mineral composition, samples were digested using a microwave-assistant closed vessel digestion system (MARS, CEM Corporation, North Carolina). Then analyzed by Inductively Coupled Plasma-Optical Emission Spectrometry (ICP-OES) (iCPA 7000 series, Thermo Scientific).

B. Determination of Biochemical properties

The fatty acid composition was analyzed by Gas Chromatography (Agilent 7489) equipped with a flame ionization detector. *In vitro* starch digestion was conducted according to Vangsøe *et al.*, (2016) with minor modifications. FTIR analysis was carried out using an FTIR Nicolet iS50 spectrometer (Thermo Nicolet, Madison, WI) equipped with deuterated triglycine sulphate (DTGS) KBr detector and KBr beam splitter.

Total phenolic content (TPC), flavonoid content (TFC), antioxidant activities, and anti-diabetic activity of the crude extract of raw and processed *A. nobilis* seed were compared with three commercial nut types: Almonds (*Prunus dulcis*), Cashew (*Anacardium occidentale*), and Pistachio (*Pistacia vera*). Further, methanolic crude extract of *A. nobilis* seeds were analyzed for their polyphenolic profile using a UHPLC system (UltiMateTM 3000, Thermo Fisher Scientific, Germany). Ten authentic phenolic standards were used for the quantification and identification purpose.

In the determination of prebiotic activity, first seeds were subjected to simulated *in vitro* digestion using synthetic gastrointestinal enzymes. Then total non-digestible portion was separated and used as the carbon source for probiotic bacteria: *Bifidobacterium animalis* subsp. *lactis* (BB-12) and *Lactobacillus acidophilus* (LA-5).

C. Animal experiment

In the animal experiment, twenty-four male Wistar rats were obtained from the Medical Research Institute (MRI), Colombo. They were housed individually in a wire-bottomed stainless-steel cages at the animal house, Faculty of Medicine, University of Peradeniya. Experimental design and ethical aspects were reviewed and approved by the Ethical clearance committee, Postgraduate Institute of Science, University of Peradeniya, Sri Lanka. Considering the experimental design, a randomized and controlled study was conducted for 4- weeks after an initial week of acclimatization period. Animals were divided into four groups with an average of 138-140g of initial body weight per group (n=6). Experimental groups were named as (1) BD-basal diet, (2) CC –basal diet added with 1% cholesterol, (3) AN- basal diet supplemented with 3% roasted *A. nobilis* seed, (4) ANC- 1% cholesterol diet supplemented with 3% roasted *A. nobilis* seed powder.

III. RESULTS AND DISCUSSION

Carbohydrates (59.48%) is the most abundant macronutrient in *A. nobilis* and seeds and it contained a considerable amount of edible oil (26.45%), protein (11.73%) and enrich in essential minerals: potassium (5838.2 µg/g), calcium (567.3 µg/g), and magnesium (1176.1 µg/g DM). This seed can be characterized as an energy-dense plant food with a cheap source of edible oil, protein, with essential minerals. According to the results, roasting and microwaving are preferable processing methods to improve the nutrient content and shelf life.

The fatty acid composition of *A. nobilis* is unique and contained a higher level of unsaturated fatty acids. The percentage of polyunsaturated fatty acids in raw and processed *A. nobilis* seed ranged from 49.4-51.5% and linoleic acid was the most abundant. The higher level of K, Ca, Mg content together with the lower level of sodium and the presence of linoleic acid (C18:2n-6) at higher concentrations can be claimed for the protective mechanism of cardiovascular diseases.

The starch fraction of the *A. nobilis* seed mainly consists of slowly digestible starch (SDS) (46.9-61.2%). With the processing method, SDS content decreased significantly. Considering the phytochemical profile, methanolic seed extracts of *A. nobilis* were rich in bioactive compounds, with significantly higher ($p<0.05$) TPC, TFC than almond, pistachio and cashew. Roasted and microwaved *A. nobilis* seed extracts showed remarkable antioxidant potential and α -amylase inhibitory activity. The phytochemical analysis highlighted the potential utilization of *A. nobilis* seeds extracts as a source of natural antioxidants for mitigating oxidative stress-related metabolic syndromes.

Results from the *in vitro* prebiotic assay reveals the ability of *A. nobilis* seed for simulating the growth of *Lactobacillus acidophilus* than *Bifidobacterium animalis*. Boiled seeds of *A. nobilis* served as a good fermentable substrate for the growth of *Lactobacillus*.

In the animal experiment, significant changes were not observed in the blood cholesterol levels and fasting glucose level among different dietary groups. However, there was a tendency to improve the lipid profile and reduce the fasting blood glucose level with the consumption of *A. nobilis* seed. The selective stimulation of *Lactobacillus* and *Bifidobacterium* spp. in the colon and the suppression of the coliform population indicates the potential prebiotic effect of *A. nobilis* seeds.

IV. CONCLUSIONS

The results highlighted the nutritional value of *A. nobilis* seed and the richness of the bioactive compounds in there. It is a good choice as a snack for better human health and the powdered seed samples may use for functional food preparation.

ACKNOWLEDGMENT

National Institute of Fundamental Studies, Kandy

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INSTITUTIONS WHERE RESEARCH WAS CARRIED OUT

1. National Institute of Fundamental Studies, Hantana Road, Kandy, Sri Lanka.
2. Department of Biochemistry, Faculty of Medicine, University of Peradeniya

INVASIVE ALIEN PLANTS FOR CONTROL OF PESTS IN AGRICULTURE: FUNGICIDAL AND INSECTICIDAL PROPERTIES OF SELECTED INVASIVE PLANTS AND MONTMORILLONITE COMPOSITES OF THEIR PESTICIDAL EXTRACTS

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Aishani Samarasinghe graduated with a B.Sc. in Biological Science stream with Second Class (Upper Division) Honours from the University of Kelaniya, Sri Lanka in 2016. Subsequently she worked as a research assistant at the Department of Chemistry, University of Peradeniya and registered for an M. Phil. degree in biological chemistry at the Postgraduate Institute of Science (PGIS). Based on her excellent progress in research studies, her degree programme was upgraded from M. Phil to Ph.D. in 2019. In 2023, she completed her Ph.D.

I. INTRODUCTION

Invasive alien plants (IAPs) cause considerable damage to ecosystem, ecological balance, irrigation resources, and agricultural and recreational sectors (Marambe *et al.*, 2010). According to the Convention of Biological Diversity, IAPs are defined as “species whose introduction and/or spread outside their natural habitats threaten biological diversity” (SCBD, 2009).

A potential new strategy for controlling invasive plants is to use these plants as resource material to generate value added products. The invasive alien plants may contain bioactive phytochemicals that may be developed into plant-based pesticides. Regarding food security, the management and control of insect and fungal pathogens is essential in crop

cultivation and storage of agricultural products. Pests are often controlled by synthetic pesticides. However, synthetic pesticides can cause problems on the environment and public health.

The eventual replacement of synthetic pesticides by plant-based pesticides produced from IAPs would resolve the problems of both the use of synthetic chemicals in controlling insect and fungal pathogens and the unregulated spreading of IAPs.

The bioactive principles, for effective application, require being transformed into a relatively more stable form that releases the active compounds slowly under defined conditions. In this context, the intercalation of active principles with montmorillonite (MMT) clays is proposed as a step towards developing effective slow-releasing pesticides. Montmorillonite (MMT) which is a phyllosilicate mineral of the subclass smectite, is composed of an octahedral layer of alumina sandwiched between two tetrahedral layers of silica. Formulations of biopesticides are likely to be biodegradable, eco-friendly and safe to use and can be produced using bioactive natural products derived from IAPs and MMT clays.

II. METHODOLOGY

Different plant parts of the IAPs *Ageratina riparia*, *Aristea ecklonii*, *Cestrum aurantiacum*, *Dillenia suffruticosa*, *Miconia calvescens*, *Mimosa pigra* and *Ulex europaeus* were dried and powdered to obtain 19 plant powders. Each plant powder was sequentially sonicated with n-hexane, dichloromethane (DCM) and methanol (MeOH).

Antifungal activity of the dried solvent extracts was evaluated against the phytopathogenic fungi, *Colletotrichum musae*, *Lasiodiplodia theobromae*, *Aspergillus niger* and

Cladosporium cladosporioides by disc diffusion assay (2 mg/6-mm disc). Bioactive compounds from plant extracts of *Ageratina riparia* and *Aristea ecklonii* were isolated by bioassay-guided fractionation. Antifungal activity of *A. ecklonii* root hexane extract against anthracnose disease on banana was determined by treating bananas (ambul variety) with plant extract to find disease incidence and the disease severity.

To check the insecticidal activity of IAPs the dried solvent extracts were tested against diamondback moth (DBM, *Plutella xylostella*) larvae and green-peach aphids (*Myzus persicae*) by leaf disc bioassays. Insecticidal activity against bruchids (*Callosobruchus maculatus*), placed on cowpea seeds, was determined by direct contact application of plant powders (0.1 g/1 g seed). Bioactive compounds from plant extract of *A. riparia* leaf were isolated by bioassay-guided fractionation.

MMT clay composites of *A. ecklonii* and *A. riparia* were prepared by intercalating plant extracts into cation-exchanged H⁺-MMT and Al³⁺-MMT to furnish *Aristea*-H⁺-MMT, *Aristea*-Al³⁺-MMT, *Ageratina*-H⁺-MMT and *Ageratina*-Al³⁺-MMT. Capacity for releasing entrapped phytochemicals of prepared clay composites and their antifungal activity (serial micro-dilution assay and TLC bioautography), insecticidal activity (micro-applicator method) and toxicity (Brine shrimp lethality assay) was evaluated.

III. RESULTS AND DISCUSSION

Several solvent extracts prepared from seven alien invasive plants using hexane, dichloromethane and methanol as organic solvents, displayed antifungal activity to varying degrees, against four fungal pathogens (*Aspergillus niger*, *Cladosporium cladosporioides*, *Colletotrichum musae* and *Lasiodiplodia theobromae*) that are important in agriculture. Of all the plants, the extracts prepared from *A. ecklonii* (root) and *A. riparia* (leaf and root) showed the most potent antifungal activity. An extract of *A. ecklonii*, functioning as a prophylactic fungicide, prevented the onset of anthracnose on banana when the latter was infected with *C. musae*; a similar observation has been made with an extract of *A. riparia* in a previous study (Rathnayake *et al.*, 2018). Two antifungal compounds, ripariochromene A and ripariochromene C were isolated from the dichloromethane-methanol (1:1) extract of *A. riparia* root by bioassay-guided fractionation. Similarly, one antifungal compound, plumbagin, was isolated from the hexane extract of *A. ecklonii* root.

The plant extracts of *A. riparia* (leaf), *A. ecklonii* (leaf), *U. europaeus* (aerial part and root), *M. calvescens* (stem-bark) and *C. aurantiacum* (leaf) demonstrated considerable insecticidal activity against green peach aphid (*M. persicae*) and diamondback moth (*P. xylostella*). An active compound present in *A. riparia* leaf was isolated and identified as methylripariochromene A which showed considerable insecticidal activity (90% mortality at 24 hours after treatment) when tested on green peach aphids. The invasive plant *A. riparia* (leaf) is a potential source for developing plant-based insecticides to combat green peach aphid and

diamondback moth infestations. The plant powders of *U. europaeus* (aerial part and root) and *M. calvescens* (leaf, stem bark and root bark) had potent insecticidal activity against cowpea bruchids (*C. maculatus*) indicating that these invasive plants are a potential source for developing plant-based insecticides for use in post-harvest storage of grain.

The bioactive extracts of the alien invasive plants *A. ecklonii* (LC₅₀, 51 ppm) and *A. riparia* (LC₅₀, 57 ppm), which are highly toxic, can be successfully intercalated into cation-exchanged montmorillonite clays (H⁺-MMT and Al³⁺-MMT) to furnish non-toxic or relatively low toxic bioactive clay composites (*Aristea*-H⁺-MMT, *Aristea*-Al³⁺-MMT, *Ageratina*-H⁺-MMT and *Ageratina*-Al³⁺-MMT), which can slowly release the trapped bioactive phytochemicals to aqueous medium at neutral pH. The clay composites displayed antifungal activity against the pathogenic fungi *C. cladosporioides* and *A. niger*. The phytochemicals released from *Aristea*-H⁺-MMT and *Aristea*-Al³⁺-MMT as well inhibited *C. cladosporioides* and *A. niger*, one of the released phytochemicals being plumbagin, a potent antifungal agent present in *A. ecklonii*. The phytochemicals released from *Ageratina*-H⁺-MMT and *Ageratina*-Al³⁺-MMT were not screened for antifungal activity but displayed considerable insecticidal activity against aphids (*M. persicae*), an important pest in agriculture.

IV. CONCLUSIONS

The present studies reveal that some invasive plants have the potential to be developed as eco-friendly low-cost botanical fungicides and insecticides. The solvent extracts prepared from the roots and leaves of *A. riparia* and the roots of *A. ecklonii* displayed considerable antifungal activity. Two antifungal compounds, ripariochromene A and ripariochromene C, were isolated from *A. riparia* root extract, while one antifungal compound, plumbagin, was isolated from *A. ecklonii* root extract. The plant extracts of *A. riparia* (leaf) and *A. ecklonii* (leaf) also displayed insecticidal properties. The active compound present in *A. riparia* leaf was identified as methylripariochromene A, which showed considerable insecticidal activity against green peach aphids. The plant powders of *U. europaeus* and *M. calvescens* had potent insecticidal activity against cowpea bruchids.

The study also found that the bioactive extracts of *A. ecklonii* and *A. riparia* can be successfully intercalated into cation-exchanged montmorillonite clays to furnish non-toxic or relatively low toxic bioactive clay composites, which can slowly release the trapped bioactive phytochemicals to aqueous medium at neutral pH. These clay composites displayed antifungal activity against the pathogenic fungi *Cladopodium cladosporioides* and *Aspergillus niger*, and insecticidal activity against aphids. The montmorillonite clay composites of *A. ecklonii* and *A. riparia* have the potential to be developed as safe plant-based slow-releasing pesticides.

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EARTH SCIENCES

WATER INGRESS IN ROCK TUNNELS, ITS CONTROL AND MITIGATION

CASE STUDY FROM UMA OYA, SRI LANKA

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I. INTRODUCTION

The water ingress that occurred during the excavation of the Uma Oya Multipurpose Development Project was a human-induced environmental, economic, and social calamity for Sri Lanka. This thesis identifies the causes of the issue, the investigations and analyses conducted to clearly define the problem, and the methods employed to complete the tunneling works without causing further significant damage to the environment and local communities. Most importantly, the thesis evaluates the effectiveness of various methods available to address these issues and provides recommendations to minimize the risks for future tunneling projects in Sri Lanka and other countries.

The thesis describes the investigations undertaken to determine how tunneling affects both surface and deep-level aquifers in the Uva Province, Sri Lanka, and examines the control measures that may be used to mitigate these effects. The study is based on the construction work of the Uma Oya Multipurpose Development Project, with particular focus on the 15 km headrace tunnel (HRT) and the Link Tunnel. The excavation of the HRT was carried out using a double-

shielded tunnel boring machine (TBM), marking the first application of a TBM in Sri Lanka.

In December 2014, the excavation of the Uma Oya HRT encountered high water ingress at the tunnel face, with a flow rate exceeding 50 l/s. At that time, it was deemed impossible to stop the ingress at the face, and the decision was made to continue excavation until the TBM could be halted in dry conditions, requiring an advance of at least 200 m (the length of the TBM). However, over the next few meters of progress, the water ingress at the face increased significantly, reaching a maximum of 450 l/s, as measured at the Parshall flume at the portal. Surface wells up to 3 km from the tunnel, where the large water ingress occurred, dried up, leading to widespread damage to buildings due to settlement, as well as the loss of agricultural crops as surface soils dried out.

It is believed that this problem could have been, and should have been, prevented through the use of alternative tunneling techniques. The aim of this study is to gain a deeper understanding of why the ingress occurred and how such potential ingress can be controlled in the future to minimize environmental impact.

The study involved continuous monitoring of domestic wells, tube wells, surface rivers, and deep boreholes in the vicinity of the tunnel during excavation. It also included detailed reporting of the geological conditions encountered at the tunnel face and those predicted ahead of it, as well as an assessment of the effectiveness of grouting works carried out to seal off water ingress during excavation.

Several future tunneling projects are planned in Sri Lanka. However, numerous past tunneling projects have also caused significant environmental impacts on local water resources. Therefore, to ensure that future tunneling projects in the country proceed without serious adverse effects on the environment and local communities, the issues related to detrimental impacts on the groundwater regime must be addressed by selecting the most appropriate tunneling methods.

II. METHODOLOGY

a. In-Tunnel Mapping

Tunnel mapping in TBM excavation is typically conducted in two phases. First, the tunnel face is mapped

daily. Then, behind the excavation, where no rings have yet been installed, the excavated tunnel is also mapped. Both of these data sources are used to develop a geological section. To ensure the creation of complete geological sections, including structural features, face mapping and probe hole data are interpreted. This allows for more effective planning and execution of grouting work in fully segmentally lined sections.

Surface Boreholes: Additional boreholes were drilled along the new alignment and near Makulella. Permeability testing was conducted using constant head tests (Lefranc method) and falling head tests (Marg method) in accordance with EN ISO 22282-2:2012. In the rock, Lugeon testing was performed following the methodology of Houlsby (1972).

b. Tunnel Seismic Prediction (TSP)

Tunnel Seismic Prediction (TSP) was conducted in accordance with the Amberg Technologies TSP 303 Plus Operation Manual, Version 1.0.10.0, dated March 2014 (Amberg Technologies, 2014). Data processing was carried out using the TSP® 303 system software, which enabled on-site seismic imaging through the analysis of P-waves and S-waves, as well as the use of 3D-Velocity based Migration & Reflector Extraction technology (3D-VMR). On some occasions, data was sent to Amberg's head office for further processing by their qualified geophysicists.

Continuous analysis was performed during excavation to compare the actual conditions encountered with those predicted by the TSP.

c. BEAM

The system was installed by the manufacturers, *Geo Exploration Technologies*, and is detailed in the *BEAM Installation Documentation UOMDP-HRT (M1685)* (GET, 2016). The equipment was configured in "integral mode," with two bolts welded onto the outer shield near the main bearings to attach the electrodes. Four pins were installed in the left-hand side wall behind the *TBM* for the outside electrode. The control box was positioned in the *TBM* driver's cabin.

The *BEAM* system operated automatically after booting, with measurements triggered through a relay controlled by the *PLC*. The current station of the face was updated every 10 seconds via the *PLC*.

d. Electrical Resistivity in Boreholes

An electrical resistivity trial was conducted at the tunnel face at Ch 1+070 in a single borehole at the 2 o'clock probe hole position, using a Wenner array with four electrodes spaced 1.5 meters apart. An equatorial dipole-dipole configuration was then performed using two boreholes at the 2 o'clock and 10 o'clock positions.

e. In-Tunnel Manometers

Manometers were installed at several locations along the tunnel to monitor pressure variations.

f. Surface Monitoring

Surface monitoring was conducted on all buildings, domestic wells, deep wells, lakes, and streams within a 300-meter zone on either side of the tunnel alignment at ground level.

g. Deep Boreholes

During tunnel construction, water levels in all deep boreholes were measured monthly along the entire tunnel. Additionally, measurements were taken weekly within a 500-meter zone behind and in front of the excavation face. In areas where high water ingress was intercepted, daily monitoring was conducted.

h. Domestic Wells

A pre-construction survey was carried out for every well within a 600-meter wide corridor. Each well was surveyed, and detailed information—such as well type, approximate age, condition, and construction materials—was recorded. Each well was assigned an individual ID number, and a base point was established, marked with permanent paint, for future water depth readings. GPS coordinates (Kandawela) were used to map the location of each well on a *GIS* map. Water levels were measured once per month over the entire tunnel length and once per week within the 500-meter zone behind and in front of the excavation face.

i. Surface Water

Where surface rivers crossed the tunnel alignment, downstream locations were selected, and Parshall flumes were installed to monitor changes in flow. The size of the flume was determined based on anticipated flow, following the guidelines of *Skogerboe et al.* (1966). In areas with typical flow rates below 5 l/s, flow was measured by timing the filling of a 10-liter bucket. Lake levels at Lake Panangala and Elamal Landa Tank were measured by establishing a benchmark on the spillway, with water depth measured below the benchmark using a tape. Readings were typically recorded once per month.

j. Building Survey

A crack survey was conducted for all buildings within the 600-meter tunnel corridor to assess structural impacts.

k. Grouting

The properties of cement grout were measured in both the laboratory and the tunnel to assess viscosity, density, and bleeding. Chemical grout was also tested in the laboratory and the tunnel to determine foaming volume and setting times at various temperatures. The setting time of polyurethane foam was defined with the initial setting occurring upon the completion of expansion, and the final setting defined as the point when the foam was stiff and firm, without indentation upon touch. Samples were mixed in accordance with the manufacturer's *Technical Data Sheet (TDS)* in clear plastic 200 ml cups.

III. RESULTS AND DISCUSSION

a. Geology and Hydrogeology

The project area is located within the Highland Complex (HC), which forms the rugged high ground occupying the central part of Sri Lanka. The majority of the headrace tunnel (HRT) trace passes through charnockitic gneiss, often interbanded with leucocratic quartz-feldspar gneiss rich in pink garnet. This region is characterized by large-scale folding and thrusting. The rock bands exhibit primary foliation related to the main deformation phase, and these bands are further refolded into open synforms and tight antiforms.

Initial concerns for the project included the impact of the interface between the Highland Complex and the Vijayan Complex (Dietler, 2011), as well as the presence of calc-silicate/marble and quartzite. However, the interface was found to have no significant impact on tunnel excavation. The calc-silicate was predominantly homogeneous and dry, with the exception of some unique hydrothermal cavities.

Excavated sections of quartzite in the tunnel caused only minor water ingress. The problematic rocks were khondalite and charnockite. Khondalite forms large storage areas for underground water and allows direct connectivity between the lower and upper aquifers due to its intense weathering. Charnockite, due to its brittle nature, exhibited open fracturing in fold structures (particularly antiforms), creating connections to overlying aquifers.

This research has demonstrated that there is hydraulic continuity within the deeply weathered khondalite over large areas. In areas where deep weathering of khondalite occurs, the upper and lower aquifers are co-joined, and lineaments and shear zones along river valleys can influence groundwater flow over vast regions (Lees and Gunatilake, 2017). However, the study concludes that there is no strong connection between surface wells and the deep aquifer in regions where charnockite outcrops.

b. In-Tunnel Investigation

The geological conditions ahead of the working face in tunnel excavation are typically predicted by analyzing geological data from surface boreholes and the tunnel face, and applying theories of geomorphology, stratigraphy, and structural geology based on regional conditions to assess trends. While this method is effective under normal conditions, it does not account for abnormal conditions, which can be catastrophic to tunnel excavation.

Tunnel Seismic Prediction (*TSP*) technology, developed by *Amberg Technologies*, was extensively utilized in the Uma Oya HRT. It proved useful, as changes in bulk modulus and Young's modulus, determined from seismic wave analysis, indicated the following:

- Low bulk modulus with high Young's modulus: fractured rock mass
- Low bulk modulus with low Young's modulus: weathered rock mass

Additionally, a change in Poisson's ratio from below 0.2 to nearly 0.3 was often indicative of water-bearing joints. However, the presence of water was not always detected, leading to the exploration of other techniques, such as *BEAM*, a resistivity-based method. In agreement with *Schaeffer*, this research also concluded that the success of electro-resistivity techniques was limited in a highly conductive tunnel boring machine (*TBM*) environment.

Other methods, such as cross-hole tomography and borehole radar, were also found to be ineffective. Given that these techniques still required probe hole drilling, which had already proven effective in identifying water, it was concluded that they were not beneficial for this project.

c. Development of Grouting Methodology

Pre-excavation Grouting: When excavation resumed in December 2015, the amount of pre-excavation grouting was significantly increased. However, this led to a distinct

reduction in the excavation's progress rate. Due to initial limitations in the *TBM*—which only allowed for probe drilling and pre-excavation grouting between the 9 o'clock and 3 o'clock positions—some major joints could not be completely sealed, and water ingress continued to increase despite post-grouting efforts in completed sections.

The intensified pre-excavation grouting carried out between December 2015 and July 2016 had limited success at points of major water ingress. This prompted the recommendation that pre-excavation grouting should be confined to practical and effective limits, with sealing efforts focusing more on post-grouting.

Despite these efforts, in areas beneath valleys of deeply weathered rock, significant water ingress persisted, causing the drying of wells, surface streams, and damage to buildings. At Ch 7+317 in April 2017, the highest recorded water ingress exceeded 500 l/s from the tunnel face.

During the suspension of excavation at Ch 7+317, modifications were made to the *TBM*, adding 18 additional probe drilling ports to cover the full 360 degrees of the tunnel face. Some of these new holes were positioned at 11 degrees and others at 15 degrees, compared to the original 7-degree ports. Before resuming excavation, a new pre-excavation grouting regime was established using microfine cement.

The new procedure involved drilling additional pre-excavation grout holes if any probe holes identified more than 20 l/min of water ingress (Garshol, 2018). This further increased the amount of pre-drilling, which subsequently slowed down the excavation rate. While the microfine cement and disposable packers were intended to speed up the grouting process and minimize the impact on excavation speed, these advantages were not fully realized. The disposable packers could not be installed in probe/grout holes with high water volume and pressure, as the water could not pass through the packer before it was sealed in place. As a result, grouting continued with pneumatic packers “to refusal,” allowing the reusable packers to be removed before excavation continued. The fast-setting microfine cements also demonstrated limited penetration, necessitating more grout holes (Spinazzola, 2018).

From the restart of excavation on 7 September 2017 until February 2018, the excavation followed the Norwegian pre-excavation grouting system, as outlined by Garshol (2018). Over 2,000 meters of drilling for pre-excavation grouting were conducted, allowing an advance of approximately 135 meters per month. This resulted in essentially a dry excavation. However, the favorable ground conditions during this period suggest that the system was not fully tested, and this intensity of grouting may not always be necessary.

d. Post-Grouting

Following the water ingress at Ch 11+200 in December 2014, a decision was made to advance the excavation and position the *TBM* in dry ground. However, due to the lack of recorded geological structure behind the segmental lining, a systematic approach to grouting could only be implemented (Wenner and Wannenmacher, 2009). This process spanned seven months, yet there was still a residual water ingress of 60 l/s upon completion.

In the subsequent full ring section (Ch 9+900 to Ch 8+906), the joints were identified and mapped during

excavation. Points of water ingress were then isolated by constructing bulkheads on either side of these water-bearing joints. Following the completion of contact grouting, rock mass grouting was conducted but limited to a few short sections totaling approximately 100 meters across the 700 meters of the full ring, which was carried out in parallel with the excavation. This approach proved effective (Rahbar et al., 2017), resulting in a significant improvement in both time lost and sealing achieved between Ch 11+200 and Ch 10+500.

In the next section of the tunnel (Ch 8+800 to Ch 8+000), where water ingress increased to approximately 150 l/s, a two-phase system was adopted. This allowed water to continue flowing within the rock mass while providing some relief holes, as outlined in the paper presented by Rahbar et al. (2017).

The research indicated that the rapid setting of microfine cement with an accelerator was beneficial for sealing leaks during post-grouting, where only chemical grouts had previously been considered. The strategy involved first installing drainage holes, then sealing leaks in the lining, and subsequently re-injecting the grouted rock mass with microfine cement. This approach effectively reduced total water ingress, thereby minimizing residual water ingress in sections that had previously undergone post-grouting.

IV. CONCLUSIONS

The following key aspects have been demonstrated by this research:

1. Systematic probe drilling, combined with pre-excavation grouting, is essential for tunnel excavation in complex hydrogeological conditions. In *TBMs*, access is required to cover the full excavation profile to achieve satisfactory results.
2. Water ingress measurements in previously excavated sections of the tunnel can identify areas where pre-excavation grouting has not yielded satisfactory results, indicating that these areas can be sealed through post-grouting. This research supports the premise that pre-excavation grouting is more efficient and effective than post-grouting. It has been demonstrated that spending two to three days at the face could save a month or more in post-grouting efforts and significantly reduce material usage. However, it is also evident that post-grouting can be conducted in parallel with excavation and is off the critical path, thereby having a minimal overall impact on the project timeline.
3. A systematic approach based on a fixed method statement is appropriate for general grouting; however, this approach may not adequately address the requirements for intense grouting. In "dry" conditions, where less grouting is necessary, this approach is typically too conservative. This research concludes that following the "active design principle" presented by Grov (2001) is more suitable, as it allows the tunneling crew to implement the design based on encountered rock mass conditions.
4. Pre-excavation grouting requirements should consider the geology at the face, the predicted ground conditions ahead as determined by probe drilling and any in-tunnel investigation techniques, the geology of the recently excavated sections, and the results of grouting in similar ground conditions observed elsewhere in the tunnel.
5. A rapid response to stop water ingress is critical to limit the effects on the surrounding environment, especially where there is a clear hydraulic connection between the upper and lower aquifers. However, in sections of impermeable rock with thin surface soils, water ingress from a single joint may not pose a significant issue. Nevertheless, the excavation must account for potential lineaments and shear zones crossing the alignment, as these may facilitate groundwater drainage over much longer distances.
6. Continuing excavation with only two probe holes in "dry" conditions does not indicate a risk of significant water ingress. If water ingress does occur, it will not necessarily have a substantial impact on the environment if treated promptly with post-grouting.
7. For ground conditions with clean open joints, pre-excavation grouting with ordinary Portland cement (OPC), supplemented with additives to improve flow and setting time—namely, superplasticizer and accelerator—is appropriate. The use of microfine cement (MFC) may not be advantageous, as its rapid setting time limits its ability to penetrate and seal effectively.
8. High pressures of 60 to 80 bar, as prescribed by Norwegian methodology (*Norwegian Tunnelling Society, 2011*), for pre-excavation grouting with cement may not be suitable. High grouting pressure only allows the grout to travel further away from the excavation if grouting to refusal is necessary, resulting in the need for larger quantities of grout to be injected.
9. Sealing with polyurethanes and other foaming chemical grouts has demonstrated limited applicability, and it is recommended that all such chemical grouting be followed by cement grout injection for long-term stability.
10. As presented by *BASF (2011)*, post-grouting is "very efficient when pre-injection has already been carried out," and that "in the end, the final target of water ingress limit will be met, although this might take time."
11. Post-grouting in highly fractured rock is best conducted behind a lining to limit grout leaks into the tunnel, necessitating detailed mapping to understand the geological structure to be grouted, thereby allowing for the limitation of the extent of the grouting.
12. Post-grouting is most effectively achieved by constructing bulkheads to limit water flow at the rock/lining interface. These bulkheads can be easily formed in segmentally lined tunnels using polyurethane grouts through pea gravel.
13. In cases of residual high water ingress, the best results have been achieved by employing drainage holes to

- redirect water away from the rock mass, facilitating the grouting of open joints closer to the excavation.
14. An overall ingress limit is essential, and if this limit is not established through pre-construction modeling, it can be realized through the use of in-tunnel manometers and monitoring deep boreholes from the surface to record groundwater recharge.

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DEVELOPMENT OF BATTERY GRADE SRI LANKAN VEIN GRAPHITE THROUGH OPTIMIZATION OF PURIFICATION AND SURFACE MODIFICATION FOLLOWED BY SCALING UP OF THE PROCESSES

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I. INTRODUCTION

Vein graphite has emerged as a potential intercalating anode material for rechargeable Li-Ion Batteries (LIBs), especially due to its low impurity level in the raw form, high crystallinity and structural ability. Sri Lanka has large reserves of natural vein graphite with comparably high purity and high crystallinity. Li^+ can be reversibly intercalated between the graphite layers while possessing a theoretical capacity of 372 mAh g^{-1} vs Li/Li^+ in LIBs (Chang et al. 2022; Nzereogu et al. 2022). Recent investigations on Sri Lankan vein graphite has shown its high potential for LIB anode applications (Amaraweera et al. 2018; Sasanka Hewathilake et al. 2017; Naranpanawa et al. 2023; Wijayasinghe, Balasooriya, and Amaraweera 2018b, 2018a).

A well-designed purification and surface modification process can significantly improve the electrochemical performance of the graphite and increase its suitability as an anode material for LIBs. At the laboratory level, scaling up of these optimized purification and surface modification processes are crucial for enabling the production of high-quality battery-grade vein graphite anode materials on a larger scale. However, this is a complex task and requires a comprehensive understanding of the underlying factors

influencing the process outcomes (Krumdick, Pupek, and Dzwinel 2016; Piccinno et al. 2016). By adopting a systematic approach at the laboratory-level, consistency and reliability can be ensured during the scaling-up of the optimized purification and surface modification processes.

Apart from the optimization and scaling-up of already invented purification and surface modification processes, novel purification methods have also been introduced to achieve high-purity vein graphite suitable for battery applications because those former methods have shown some limitations in achieving a high carbon content of over 99.9%. Furthermore, novel methods for structural modification of graphite have also been explored to produce exfoliated or expanded graphite, evaluating their applicability in improving the electrochemical performance of LIBs. Moreover, the stability of the anode made of vein graphite has been investigated in order to improve performance, particularly in terms of achieving high Coulombic efficiencies and optimizing certain factors to determine the effect on battery performance.

In this study, the main objective is to develop battery-grade graphite from Sri Lankan natural vein graphite for the anode application in Li-ion rechargeable batteries through optimizing the existing purification and surface modification processes, followed by achieving the optimum scale-up at the laboratory-level.

II. METHODOLOGY

The vein graphite samples for this study were obtained from the Kahatagaha-Kolongaha mine, which is one of the major graphite mines in Sri Lanka. Raw graphite (NPG morphological variety) samples were crushed into small chips and sieved through a 1 mm mesh size plastic sieve. The powdered samples ($< 1 \text{ mm}$) were ground for one to two minutes in a vibratory disk mill (Shimadzu, Japan) to obtain the very fine powder samples. Then, the fine graphite powder was sieved using a mechanical sieving setup and the undersized fractions below $53 \mu\text{m}$ of fine graphite powder were collected. Collected fine graphite powder ($< 10.00 \text{ g}$) was subjected to purification methods.

a. Sample Preparation for Batch Scale Scaling-up Optimized Purification Method at Laboratory-Level

Batch scale scaled-up purified graphite were prepared using the HCl acid-leaching method for substantial amounts (< 100.00 g) of raw graphite and subjected to an optimized purification method of batch level scaling-up at laboratory level. Batch level scaling-up refers to increasing the batch size by a factor of ten or greater the size of the initial batch or initial sample. Laboratory level refers to the experimentation and testing of a process or material at a small scale, typically in a laboratory setting. Initial investigations have been carried out in batch mode with the confirmation of statistical and Life Cycle Assessment studies.

b. Sample Preparation for Batch Level Scaling-up Optimized Surface Modification Method at Laboratory-Level

Surface modified graphite samples were prepared using HCl acid-leached graphite (< 10.00 g) that had been prepared using purification methods and subjected to surface modification methods. Batch level scaled-up surface modified graphite samples were prepared using considerable amounts (< 100.00 g) of purified graphite and subjected to an optimized surface modification method. Initial investigations have been carried out in batch mode with the Life Cycle Assessment studies.

c. Sample Preparation for Structural Modification Methods

Structural modified graphite samples were prepared using raw graphite that were cut into graphite rods (6 cm x 1.5 cm x 1.5 cm) using a rock cutting machine (Shimadzu, Japan). Then, the graphite rods were subjected to structural modification.

Prepared mineral samples were characterized by powder X-ray Diffractometer (XRD), Fourier Transform Infrared (FTIR) Spectroscopy, Inductively Coupled Plasma Mass Spectroscopy (ICP-MS), Raman Spectroscopy, Scanning Electron Microscopy (SEM), X-ray Photoelectron Spectroscopy (XPS), Particle Size Analyzer (PSA) and Brunauer-Emmett-Teller (BET).

These processes utilize the doctor-blade tape casting method to fabricate graphite anode electrodes. Electrochemical characterization was performed on assembled LIB coin cells in Ar-filled glove box, including Galvanostatic charge-discharge analysis, Cyclic Voltammetry (CV) analysis and Electrochemical Impedance (EIS) analysis.

d. Optimization of fabrication process of anode

The effect of the slurry with different compositions of active material (AM), conducting material (CM), and PVDF binder were investigated with 2.0 ml NMP solution to cast thin tapes of about 100 μm thickness on Cu foil. After optimizing the relevant composition of the tape casting slurry used for the anode fabrication process, the thickness of the coated electrode was also optimized. The thickness of the

coated electrode was in the range of 50 μm , 100 μm , and 250 μm .

III. RESULTS AND DISCUSSION

The major findings in this research project can be presented as follows.

Under the study of optimization of purification methods for Sri Lankan natural vein graphite, the investigations were carried out with already invented purification methods. The HCl acid leaching method was simple, energy-efficient but effective against for certain other impurities. Considering these aspects, the HCl acid leaching method was chosen for further investigation and optimization. Optimization parameters were determined as 10.0 vol.% HCl concentration, 75 minutes of leaching time, 60 °C temperature with a solid: liquid ratio of 3:20.

The study also explored the scaling-up of the HCl acid leaching method at the laboratory level. This involved attempts to narrow the gap existing between preliminary laboratory experiments and industrial level production. It emphasizes the complexity and case-specific nature of scaling up of chemical processes for graphite purification. The outcome of the present study shows that the performance of the scaled-up graphite samples is comparable to that of the initial laboratory sample in terms of capacity, rate capability, and cycling life for LIBs. The study suggests that simply applying proportional rules for masses and volumes is not sufficient for batch-level scale-up. To address this challenge, the study proposes the use of linear regression models and the design of experiments to establish the cause-and-effect relationships between process parameters and the purity of the graphite samples. It concluded by discussing the development of a scale-up framework using laboratory level data and life cycle assessment studies, which aims to improve scale-up efficiency and assess the energy consumption of industrial scale production.

Additionally, the present study has introduced a novel combined purification process by combining acid leaching and alkali roasting processes. Here, 5.0 vol.% for HCl and 25.0 vol.% for NaOH have been used for this novel combined acid leaching-alkali roasting process developed for achieving high purity of 99.9% carbon content, which is acceptable for the anode of the LIB.

The study of optimization of surface modification was carried out because of the high potential shown by surface modified vein graphite as an anode material in LIBs. Mild oxidation, particularly the NO and NS methods using HNO_3 and $(\text{NH}_4)_2\text{S}_2\text{O}_8$, respectively as oxidants, demonstrated successful surface modification while preserving the crystal structure. Under this study, the optimization of surface modification by chemically mild oxidation method using HNO_3 was investigated. Consequently, 50 ml of 69.0% HNO_3 was determined as the optimized condition for scaling up the selected surface modification process.

This study further investigated the scaling-up of surface modification process at the laboratory level, addressing the

limitations of laboratory conditions and facilities. In order to overcome these limitations, the scaling-up process of the NO method was explored and a framework was developed based on laboratory data. This framework supports better production results and enables comparative life cycle assessments. The scaled-up graphite samples show similar performance to that of the initial laboratory sample in terms of characteristics and performance.

The quenching process which enhances the purity of graphite by peeling it into thin sheets and inserting small molecules into the interlayer spaces, was also studied. The choice of quenching solutions influences the quality of the resulting graphite sheets. The anodes for LIB fabricated from this modified graphite exhibit improved electrochemical performance, with high discharge capacity and Coulombic efficiency.

Finally, the stability of the LIB anodes fabricated from raw vein graphite was investigated to enhance performance, particularly in terms of achieving high Coulombic efficiencies. Among the tested samples, the one labeled as 85-10-5 (tape casting slurry consisting of 85% of graphite, 15% of carbon black, and 5% of PVDF) exhibited the highest retained capacity (92.82%) and the least irreversible capacity loss (1.7 mAh g^{-1}), indicating superior performance. Additionally, the thickness of the fabricated electrode by tape casting was optimized and a $100 \mu\text{m}$ thickness was demonstrated to be the optimum thickness for the battery performance.

IV. CONCLUSIONS

This study has made significant contributions to the optimization and scaling-up of purification and surface modification processes for vein graphite. It highlighted the capability of industrial level production of the graphite-based materials for those variety of potential applications, including LIBs. Further research and development efforts are needed to improve the cost-effectiveness, environmental friendliness, and performance enhancement of these materials in order to enable final industrial-scale production and widespread use.

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IMPACT OF COVID-19 ON SECONDARY SCHOOL EDUCATION: LESSONS LEARNED FROM KARAVEDDY EDUCATION DIVISION, JAFFNA, SRI LANKA

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- i. To investigate the strengths and weaknesses of Secondary School education during COVID-19.
- ii. To investigate the opportunities and threats of Secondary School education during COVID-19.
- iii. To compare the student's performance during the pre-and post-COVID-19 pandemic.
- iv. Propose the possible recommendations for future calamity.

II. METHODOLOGY

B. Abiramy graduated in 2018 with a B. A Special in Geography with Second Class Upper Division from the University of Peradeniya, Sri Lanka. Soon after her graduation, she worked as an Assistant Lecturer at the Department of Geography, Faculty of Arts at the University of Peradeniya, Sri Lanka. In 2023, she completed her M.Sc. by research from the Postgraduate Institute of Science (PGIS), University of Peradeniya, Sri Lanka.

I. INTRODUCTION

The COVID-19 pandemic in Sri Lanka is part of the global pandemic, affecting various sectors, including education. Sri Lanka's school education system shifted from classroom-based education to distance learning education during the COVID-19 pandemic lockdown period. Distance learning modalities cannot be uniformly applied nationwide as children have varying levels of access to technology infrastructure in the country. Even though free education assures equal rights to all students, the COVID-19 pandemic seems to cause inequalities in the education system (Sandepani et al., 2021). Despite these challenges, the pandemic also provides opportunities in some contexts for developing more flexible learning solutions that better utilize distance learning and digital tools. Therefore, it is important to identify the positive and negative aspects of distance learning during school closures. The impact of this epidemic was strongly felt by the General Certificate of Education (G.C.E.) A/L (Advanced Level) and O/L (Ordinary Level) candidates. This study examined the impacts of COVID-19 on secondary school education in the Karaveddy Education Division (KED) in the Jaffna district, Sri Lanka.

The objectives of the study were,

This study was conducted in 16 schools within the KED of the Jaffna district, Sri Lanka. Primary data for the research were obtained from KED students and teachers. This study employed a questionnaire survey to collect data regarding the research problem on the positive and negative impact of distance learning during the COVID-19 pandemic lockdown. Secondary data were collected from various sources. The students' O/L and A/L results were collected directly from the Vadamaradchy Zonal Education Office. By quantitative approach, descriptive statistical tools were used to analyze and compare student performance before and after COVID-19 based on O/L and A/L results. In addition to that, Box-Whisker plots were used for graphical presentation. Positive and negative impacts were analyzed using SWOT (Strengths, Weaknesses, Opportunities, and Threats) metrics.

III. RESULTS AND DISCUSSION

Results of the questionnaire survey with students and teachers indicate that teaching-learning methods such as Television broadcasts, YouTube videos, and distributing learning materials played a major role during school closure. The respondents' continuation of distance education using their existing learning resources was considered a strength. Common problems associated with online education in general include the limited availability of the internet, the slow speed and high cost of the internet bills, and the lack of interaction between students and teachers. Financial problems were also the major challenge faced by school students. Due to their poor economic background, 59.2% of students reported that internet services were too expensive for regular online connectivity. Teachers identified several key problems while engaging in distance and online teaching, including lack of student engagement (65.7%), lack of teacher training for conducting online classes (51.9%), and students' irregular attendance in online classes (75.5%).

On a positive note, most people in the country are reluctant to adopt new technological methods in education. But with the challenges that arise with the COVID-19 pandemic, it was started to facilitate. In addition, it also gave them opportunities to connect with teachers and peers from far-away places on one platform (86.2%) and provide a good experience to face future calamities (80.8%) where schools can continue education without interruption during this kind of school closure and 80.7% of teachers identified that it has upskilled new technology.

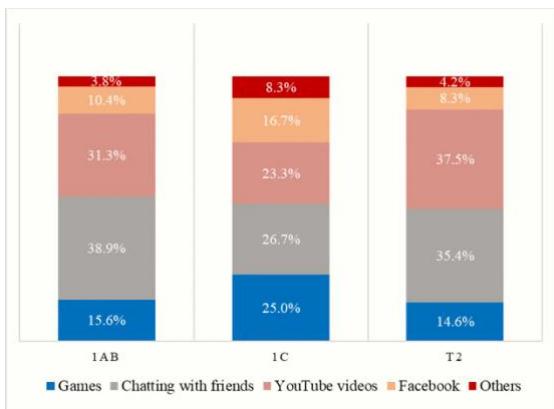


Fig. 1: Purpose of using smartphones/computers during COVID-19 school closure, apart from learning

This study highlights some key points concerning the threats of online learning. One of the major threats was health-related issues such as eyesight issues, headaches, and back pain. 57.10% of students were concerned that they had too much screen time. Moreover, for the learners who were less determined and lacked self-motivation, the chance of them getting distracted was very high. According to Figure 1, students have engaged in playing games, using Facebook, chatting with friends, watching YouTube videos, and some created TikTok videos.

This study clearly illustrates that students' final academic performance has been notably influenced by their exposure to online learning during the pandemic lockdown. Especially 2020 A/L students' results declined. The G.C.E.(A/L) 2020 exams began under strict COVID-19 health guidelines. However, O/L students' academic performance was quite well. Satisfactory academic performance among O/L students is a positive sign.

IV. CONCLUSIONS

The study reveals the impacts of the COVID-19 pandemic lockdown on senior secondary students' educational performance. While KED students demonstrated resilience by continuing their distance education with available resources, many encountered significant challenges, including financial constraints, limited digital skills, and a lack of training. Despite fostering opportunities for hybrid learning and up-skilling in technology, online education posed threats like health issues and device addiction. However, the academic performance of A/L students declined post-pandemic.

Overall, while online learning can be effective to a certain extent, the majority of KED students were hindered from fully accessing its benefits.

This study recommends the following suggestions to be prepared for future crises.

- i. School teachers and students should get the proper training to handle online classes without interruption to face similar situations in the future.
- ii. All the teachers in this KED were using the free version of Zoom, in the middle of the classes the whole class had to re-join to continue the lesson. Schools should take necessary action to continue classes without interruption.
- iii. An island-wise survey is needed to prioritize the most vulnerable students who gained the minimum learning experience during COVID-19. It will provide equal opportunities for every student in Sri Lanka to continue their learning even during the disaster situation.

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ENVIRONMENTAL SCIENCE

INVESTIGATION OF GENETIC DIVERSITY OF CYANOBACTERIA IN SOME SELECTED EXTREME ECOSYSTEMS OF SRI LANKA WITH THEIR TAXONOMICAL IDENTIFICATION AND NUTRIENT PROFILING

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I.INTRODUCTION

Sri Lanka is a tropical island with a remarkable faunal, floral and microbial diversity. A wide range of ecosystems within and surrounding the country facilitates diverse habitats for ubiquitously occurring cyanobacteria, thus cyanobacteria being a major contributor of the biodiversity of Sri Lanka. Producing rapid responses to environmental fluctuations with effective morphological, biochemical, and physiological adaptations is a key feature of cyanobacteria for their successful survival in every ecosystem. Cyanobacteria communities from extreme ecosystems may have uniquely adapted and may be diverse by morphology, physiology and genetic composition. Therefore, the extreme ecosystems are

expected to bear a unique, endemic and novel cyanobacteria diversity which may not be found elsewhere. However, inadequate research on the cyanobacteria occurrence and diversity in Sri Lankan extreme ecosystems highlight the necessity of conducting broad diversity studies on cyanobacteria to generate new scientific knowledge, identify their current status of conservation in extreme ecosystems, and implement suitable conservation approaches.

Productivity limit, food scarcity and nutritional inequality are more prevalent, seeking immediate sustainable solutions through high-quality food alternatives. Naturally occurring, nutrients rich cyanobacteria have been proved to be more promising in nutritional applications. Unique adaptations of cyanobacteria from extreme ecosystems would result in exceptionally high potential in nutritional applications. It is important to identify and improve efficient native cyanobacteria as commercial products within the country rather than utilizing imported, non-native/exotic strains with a potential threat to the local natural environments. Moreover, the nutrition-based value-addition of extreme cyanobacteria would suggest sustainable approaches to mitigate the prevailing global nutritional issues. Therefore, this study mainly focused on the investigation of the genetic diversity of cyanobacteria in some selected extreme ecosystems in Sri Lanka as a basic step for conservation, and the identification of their value-added potential towards nutrition-based industrial applications.

II.METHODOLOGY

A. Sample collection

Water sampling was carried out from four selected ecosystems in Sri Lanka, namely, salt marshes and salt pans, mangroves, hot water springs and lagoons. Three water samples were collected from each location. The temperature, the pH and the conductivity of water samples were measured

using the thermometer and the digital portable multiparameter respectively. The collected water samples were stored in pre-sterilized 500 mL glass culture bottles until they were ready for laboratory culturing.

B. Cyanobacteria isolation from environmental samples

Each water sample was filtered through 20 µm mesh size planktonic net. Prepared samples were cultured in 40 mL of BG-11, GO and ASN-III media in sterile 100 mL conical flasks, under laminar flow chamber and were incubated under 2000 lux light intensity with constant illumination at 25 °C and 40 °C on shakers with the shaking speed of 80 rpm. Cyanobacteria were isolated through regular microscopic observations and subsequent plate culturing and pure monocultures were stored and maintained at 25 °C and 40 °C, under 2000 lux light intensity.

C. Morphological identification of the isolates

Pure isolates were observed under the compound light microscope and morphologically identified based on the morphological characteristics described by Desikachary 1959, McGregor 2013 and McGregor 2018. Cyanobacteria diversity was evaluated in terms of species richness and relative abundance using Shannon-Wiener index and Simpson's diversity index respectively.

D. Molecular characterization of isolates and phylogenetic analysis

Molecular characterization was carried out for 52 morphologically distinct isolates representing all four ecosystems. DNA extraction was carried out for fresh cyanobacteria biomass and the PCR amplification of the 16S rRNA gene region was carried out using cyanobacteria specific primer pairs. The amplified PCR product purification and bidirectional DNA sequencing of 16S rRNA gene region were performed by the Macrogen, South Korea, with amplified samples of respective forward and reverse primers. Sequence alignment and required minor editions were carried out using BioEdit software version 7.2.5. Aligned DNA sequences were compared with the reference sequences in the National Center for Biotechnology Information (NCBI) database using Nucleotide Blast option and the strains were identified at the molecular level. Eleven phylogenetic trees were constructed based on the family of the identified strains using Molecular Evolutionary Genetics Analysis (MEGA) version 11.0.13 and the genetic diversity was evaluated using 16S rRNA based phylogenetic analysis.

E. Value addition of cyanobacteria for nutrition-based applications

All cyanobacteria isolates were cultured in 1/5th of BG-11 and GO media at pH 7.5 in 100 L fish tanks under normal greenhouse conditions. The fresh biomass was harvested at their maximum and steady biomass concentration. Filamentous strains were harvested using continuous filtration while unicellular strains were flocculated with FeCl₃ and harvested through continuous filtration. The harvested fresh biomass was oven dried at 55 °C and the dry biomass was made in to a fine powder using a mortar and

pestle. The powdered dry biomass was stored in the -80 °C freezer for further analysis. The powdered biomass was then used to analyze the total carbohydrate content (% w/w) (Dubois' method), total protein content (% w/w) (Lowry method), and macro and micro mineral profiles using Inductively Coupled Plasma Optical Emission Spectroscopy. Efficient cyanobacteria isolates were further tested for the total pigment content and the phytochemical properties in terms of total phenolic content and ferric reducing antioxidant power. Data were statistically analyzed and compared using One-way ANOVA Tukey Pair-wise comparison ($p=0.05$) using Minitab 17 (2016) software version 2.0.

F. Establishment and registration of the NIFS- Sri Lanka Culture Collection

A large growth chamber facilitating the storage of 1000 liquid cultures in conical flasks (250 mL) was constructed with required culture conditions. It was established in the cyanobacteria culture laboratory of Microbiology and Soil Ecosystems research project at the National Institute of Fundamental Studies (NIFS), Kandy. The conditions tested and the protocols improved during the study for isolation, culturing, storage, identification and short- term and long-term preservation of cultures were adopted as procedures to maintain the culture collection. The culture collection was registered in the WFCC-MIRCEN World Data Center for Microorganisms (WDCM), under the name of NIFS-Sri Lanka Culture Collection (NIFS-SLCC) and the registration number of WDCM 1245 in 2021.

III.RESULTS AND DISCUSSION

Morphological characterization of the isolates revealed a rich diversity of morphologically distinct cyanobacteria, allowing the identification of 19 different genera namely; *Anabaena*, *Cyanosarcina*, *Fischerella*, *Geitlerinema*, *Gloeocapsa*, *Leptolyngbya*, *Limnothrix*, *Lyngbya*, *Microcystis*, *Nodosilinea*, *Nostoc*, *Oscillatoria*, *Phormidium*, *Pseudanabaena*, *Spirulina*, *Synechococcus*, unidentified genus of Chroococcales, unidentified genus of Nostocales and unidentified genus of Oscillatoriales. The highest cyanobacteria richness was recorded in salt marshes/pans ecosystem while the lowest was recorded from lagoons. The calculated values of Shannon-Wiener index and Simpson's diversity index implied that both mangrove and lagoon ecosystems had lower cyanobacteria diversity.

DNA sequencing of 16S rRNA gene region of 52 morphologically distinct cyanobacteria further confirmed the rich cyanobacteria diversity in extreme ecosystems in Sri Lanka, with the presence of 17 cyanobacteria genera namely, *Nodosilinea*, *Leptolyngbya*, *Nostoc*, *Westiellopsis*, *Phormidium*, *Calothrix*, *Chroococcidiopsis*, *Oxynema*, *Neowollea*, *Marileptolyngbya*, *Geitlerinema*, *Pseudanabaena*, *Gloeocapsa*, *Jaaginema*, *Hapalosiphon*, *Mastigocladus* and *Cyanosarcina*. *Leptolyngbya* was the most abundant genus identified in all the four ecosystems.

For the first time, this study unveils the presence of 14 previously unidentified species/genera/families of cyanobacteria in extreme ecosystems of Sri Lanka,

highlighting the unique cyanobacteria richness in Sri Lankan extreme ecosystems, and these findings mark a significant contribution as all these cyanobacteria represent novel records within the Sri Lankan ecosystems while some are novel/rarely reported in the world. They were *Phormidium irriguum*, Desertifilaceae cyanobacterium, *Nodosilinea chupicuarensis*, *Nostoc elgonense*, *Oxynema aestuarii*, *Neowollea manoromense*, *Gloeocapsa gelatinosa*, *Cyanosarcina* sp., *Pseudanabaena lonchoides*, *Jaaginema* sp., *Chroococcidiopsis thermalis*, *Chroococcidiopsis cubana*, *Marileptolyngbya sina*, and *Leptolyngbya boryana*

Nutrition-based value-added potential of these uniquely adapted native cyanobacteria was found to be more promising with nine strains with more than 50% of total protein contents, *Nostoc* sp. being the highest (75.5%) which is among the highest protein contents recorded so far all around the world. The above protein content of *Nostoc* sp. was comparable to *Spirulina* with up to 78% of content. The highest total carbohydrate content was recorded in *Leptolyngbya* sp. (54.0%) followed by seven more strains with more than 40% of total carbohydrate contents and sixteen more strains with more than 30% of total carbohydrate contents. Sodium was the most abundant in many isolates, whereas Ca or Mg was the most abundant in others. Many strains with more than 30% of total carbohydrate contents were rich sources of sodium mineral. Interestingly, the recorded Ca contents in many isolates exceeded the amounts recorded for *Spirulina* (1300-14000 mg kg⁻¹) which was comparable to the Ca amounts in milk. The iron concentration was the highest among other studied micro minerals in several tested strains. The majority exceeded the general iron contents found in cereals and grains (25-80 mg kg⁻¹), indicating that they are more suitable for development or incorporation into meals as a natural iron supplement.

Cyanobacteria strains showed a significantly higher production of chlorophyll a compared to chlorophyll b. Fifteen strains were recorded with more than 1 µg mg⁻¹ of chlorophyll a while many were reported with less than 1 µg mg⁻¹ of chlorophyll b content. The majority of the higher chlorophyll a and b producers were from hot water springs, implying their physiological adaptations to tolerate long term irradiance and higher temperatures in hot water springs. Compared to total chlorophyll content, the carotene production was significantly lower in many strains. Phycobiliproteins were the highest in production, contributing to the most part of the total pigment concentration of many strains.

Total phenolic content ranged in between 208.8 µg g⁻¹ and 4424.5 µg g⁻¹ with greater total phenolic contents for *Nostoc* sp. and Desertifilaceae cyanobacterium compared to previously studied freshwater cyanobacteria in Sri Lanka. The highest ferric reducing ability was recorded in *Chroococcidiopsis cubana* while *Nostoc* sp. and *Phormidium* sp. were also recorded with significantly greater ferric reducing ability.

Based on the developed and established procedures, the NIFS-Sri Lanka Culture Collection (NIFS-SLCC) was established and is being maintained with 248 isolates. Registration of the culture collection at WFCC-MIRCEN World Data Center for Microorganisms (WDCM) enhanced national and international level recognition of the culture collection. The latest statistics of the World Directory of Culture Collections revealed that there are nine culture collections which have been registered from Sri Lanka. Among them, the NIFS-Sri Lanka Culture Collection (NIFS-SLCC) is the first and the largest cyanobacteria specific culture collection established and registered so far, in Sri Lanka.

IV. CONCLUSIONS

In conclusion, this study could provide strong recommendations of native strains isolated from extreme ecosystems as better alternative natural sources of proteins, easily digestible carbohydrates, macro minerals, micro minerals, pigments and other phytochemical compounds with promising antioxidant properties and nontoxic coloring properties to be utilized in nutrition-based applications, aiming the eradication of prevailing local and global scale malnutrition and ensuring food security and nutritional equality. The preservation and conservation of these valuable microbial genetic resources have already been initiated by this study. The knowledge generated by the study further improves the awareness of cyanobacteria diversity in Sri Lankan extreme ecosystems to establish future conservational measures. The records of significant number of previously unidentified/rare species in these extreme ecosystems implied the knowledge deficiency on the microbial diversity of these extreme ecosystems, highlighting the necessity of immediate further research in these ecosystems.

ACKNOWLEDGMENT

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AWARDS

- [1] Research Excellence Award 2023 (Third place) in the category of Research Assistant, National Institute of Fundamental Studies, Sri Lanka
- [2] Best Paper Award under the theme of Sustainable Agriculture and Environments (Food Science): International Research Conference of SLTC Research University 2023 (IRC2023)
- [3] Visiting Research Scholarship (Training Program on Biogas Production, Power generation and Vehicular Application conducted by the Indian Institute of Technology, Delhi) awarded by the Ministry of External Affairs, Government of India in 2019.
- [4] Winner-Third place - Three-Minute-Thesis Competition organized by the NIFS-Young Scientists' Association (YSA), National Institute of Fundamental Studies, Kandy, Sri Lanka in 2018

THESIS REFERENCE

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INSTITUTION WHERE RESEARCH WAS CARRIED OUT

National Institute of Fundamental Studies, Hantana Road, Kandy, Sri Lanka.

EFFECT OF NATURAL AND ANTHROPOGENIC ACTIVITIES ON BIOTIC AND ABIOTIC FACTORS OF RAWAN-OYA TRIBUTARY OF MAHAWELI RIVER IN KANDY DISTRICT, SRI LANKA

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I. INTRODUCTION

Surface waterways are declining at a much faster rate than terrestrial ecosystems. Rivers exchange materials, nutrients, and energy, in a mutual manner with the immediate environment. Consequently, the water quality, sediment characteristics, and biological communities reflect the characteristics of the surrounding environment. These ecosystems are extremely important since they provide freshwater and habitats not only to people but also to much of life on the planet; however, merely a minute amount (0.006%) of the world's freshwater is present in streams and rivers (Malmqvist et al., 2002).

The stream ecosystem functions are directly associated and interdependent on their physical, chemical, and biological factors (Sandin and Solimini, 2009). The quality of water is described by its physicochemical properties and biological composition. Streams can get polluted due to natural and anthropogenic activities. Increasing anthropogenic stresses are the major cause that alter water

quality and induce the degradation of the stream ecosystem. Continuous monitoring of physicochemical parameters and microbiological composition as well as emerging contaminants in water is required for the conservation of stream ecosystems and inhabiting fauna.

The primary objective is to study the effect of biotic and abiotic factors on water quality, and fish distribution and abundance of Rawan-Oya Tributary of the Mahaweli River, in the Kandy District during dry and wet seasons. The specific objectives are:

- i.To assess the contribution of the riverine community towards the water quality of the stream
- ii.To study and compare the physicochemical properties of water in selected sites from the catchment area to the downstream of the Rawan-Oya Tributary
- iii.To determine the faecal contamination along the river in nine sampling sites representing five habitats: pristine, agriculture, rural, semi-urban and urban
- iv.To study the fish assemblage structure, and habitat preferences along the tributary and determine how biotic and abiotic factors contribute to the diversity, abundance and distribution of fish fauna of the Rawan-Oya Tributary
- v.To examine the level of microplastic contamination of stream water and sediments along the Rawan-Oya Tributary and classify the MPs detected in the samples based on the physical forms and the polymer type

II. METHODOLOGY

A. Study Site

The Rawan-Oya is a perennial stream, and one of the major water sources of the Polgolla Reservoir located in Kandy, Sri Lanka. Rawan-Oya starts from Hunasgiriya Mountain (1,400 m) located in the Campbell's lane forest reserve. Nine sampling points signifying the human activities adjacent to the

waterway as pristine, agricultural, rural, semi-urban, and urban were selected for the sample collection. Water samples were collected over 28 months from June 2019 to March 2022 at one-month intervals.

B. Community Survey

A semi-structured questionnaire survey was conducted to assess the sanitation, hygiene, and water-handling practices and to elicit basic demographic details of the households in the vicinity of the tributary.

C. Physicochemical Properties of Water

Water temperature, pH, dissolved oxygen (DO), electrical conductivity (EC), total dissolved solids (TDS), velocity and stream flow were measured using portable meters. Phosphate (PO_4^{3-}), nitrate-nitrogen (NO_3^- -N), nitrite-nitrogen (NO_2^- -N), ammonia nitrogen (NH_3 -N) were measured at the laboratory using the spectrophotometers. The biological oxygen demand (BOD_3) was measured with the Winkler method.

D. Faecal Indicator Bacteria Tests - Total Coliforms (TC), Faecal Coliforms (FC) and Faecal Streptococci (FS)

Water samples were filtered through membrane filters (0.45 μm pore-size), and placed on Petri-dishes, containing M-Endo, M-FC and KF-streptococcus agar. M-Endo and M-FC plates were incubated for 36 hr at 35 °C and 44.5 °C, respectively. KF-streptococcus agar plates were incubated for 48 hr at 35 °C. Confirmation tests were performed using brilliant-green bile broth for TC and FC, Indole test was done for *E. coli*. FS was confirmed by the growth in BHI broth and the colour change of BEA slant. Sources of FIB were analysed using FC: FS ratio.

E. Library Independent Molecular-Based Microbial Source Tracking (MST) Tests

Water samples ($n = 171$) were filtered through 0.45 μm sterile membrane filters. DNA was extracted from membrane filters using the gDNA Tissue Miniprep System. The Bac32 assay was performed for general *Bacteroides spp*, while human and cattle faecal contamination was tracked using *Bacteroides* HF183 and CF193 markers, and dog faecal contamination was tracked using *Faecalibacterium* ED-1 marker. PCR amplifications were performed in Applied Biosystems- Veriti Thermal Cycler. PCR products were visualized on a 1.5% agarose gel with Ethidium bromide, and using a 50 bp DNA ladder (GeneRuler 50 bp DNA Ladder, Thermo Scientific, USA).

F. Fish Sample Collection and Identification

Samplings were conducted by two netting methods: a cast net with 1 cm mesh size and 3 m in diameter, and a dip net

with 0.3 cm mesh size and 0.3 m. The collected fish samples were identified *in situ* to species level by evaluating their morphometric and meristic characteristics. Fish species richness (S) and the Shannon diversity Index (H') were calculated. A canonical correlation analysis identified the relationship between fish assemblage structure and water quality parameters.

Microplastic Analysis of Stream Water and Sediments eighty liters of surface water from each site were sieved serially through 5000 and 65 μm steel mesh sieves.

Riverbank sediment samples were dissolved in a saturated NaCl solution. the supernatant was sieved serially through a 5000 and 65 μm steel mesh sieves. The residues collected onto the 65 μm mesh were subjected to wet peroxide oxidation and density separation.

Afterward, microplastics extracted onto the membrane filter (0.45 μm) were enumerated using a dissecting microscope. Fourier transform infrared spectroscopy (FTIR) was used to identify polymer types (Kapukotuwa et al., 2022). Generalized linear model with a negative binomial distribution was used to determine whether the density of microplastics varied by site for water and sediment separately. Pairwise comparisons were used to determine differences along the urban-rural gradient.

III. RESULTS AND DISCUSSION

The results indicated that the community receives many benefits from the tributary, including water for drinking, bathing, washing, and agriculture. Nevertheless, community activities such as dumping garbage, directing greywater and blackwater etc., damage the stream ecosystem.

The optimum physicochemical and microbial water quality were recorded in the pristine area. The highest nutrient pollution level was observed in agricultural areas. Bacteriological tests revealed substantial variability in TC, FC, and FS counts spatially and temporally. The TC concentration of stream water ranged from 2.7×10^2 – 1.6×10^5 CFU/100 mL, FC counts varied from 16 – 3.2×10^4 CFU/100 mL and the FS concentrations fluctuated between 5.4×10^2 – 6.2×10^3 CFU/100 mL throughout the study period in nine sampling sites along the stream. A significant increase in FIB level was observed from the pristine to urban gradient ($p < 0.05$) (Fig. 1).

According to the MST, urban sites indicated the highest human-faecal contamination. The microbial quality of stream water did not meet the standards and guidelines for drinking. Only the pristine area contained acceptable microbial levels for recreational activities. High FIB concentrations during the rainy period compared to the dry season were observed in most sites. Moreover, water temperature, BOD, TDS, nitrite and phosphate demonstrated a significant correlation with FIB (r^2 ; $p < 0.05$).

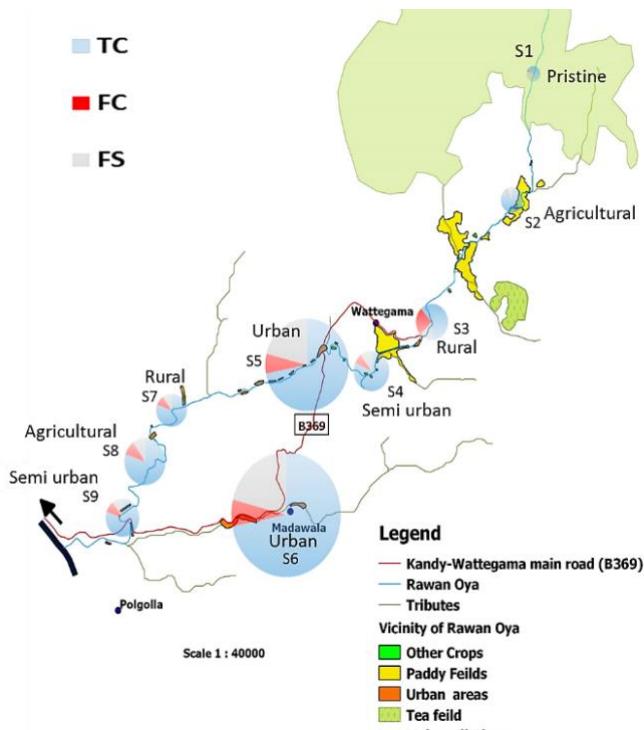


Fig. 1: Map of the Rawan-Oya Tributary of Mahaweli River illustrating the distribution patterns of total coliform (TC), faecal coliform (FC) and faecal streptococci (FS) in nine sampling sites from June 2020 to March 2022.

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According to the fish survey, twenty fish species belonging to 17 genera and 11 families were recorded. The family Cyprinidae was the most dominant, followed by Poeciliidae and Danionidae. Fish species *Dawkinsia singhala*, *Garra ceylonensis* and *Schistura notostigma* are endemic to Sri Lanka. The characteristics of the habitat strongly influenced the fish assemblage structure. The species richness (S) and diversity (H') in pool, run and riffle habitats were reported as 19, 19, and 5 and 2.5, 2.3 and 1.5, respectively. There was no difference in fish diversity and richness between dry and wet seasons (GLM; $p > 0.05$). *Schistura notostigma* was the most influential species at high altitudes (canonical coefficient = 3.7) and was associated with high DO content, low levels of nutrients, and FIB levels. *Poecilia reticulata*, and *Devario malabaricus* were common in sites with high BOD, EC, high faecal and nutrient pollution and demonstrated high tolerability toward the reduced water quality conditions. The fish assemblage structure of the tributary exhibited relationships with the habitat characteristics and the water quality parameters. (Figure 2).

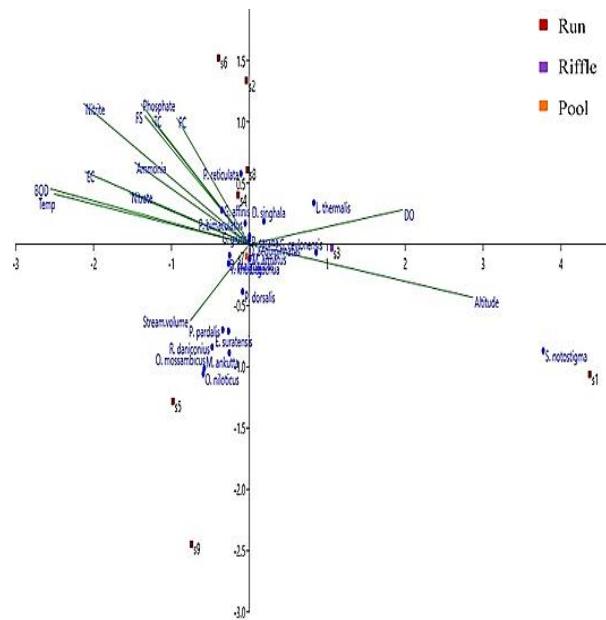


Fig. 2: Canonical correspondence analysis map of fish species, the sites, and the environmental variables

The microplastic analysis confirmed that all tested stream water and riverbank sediment samples were contaminated with microplastics. Four different physical forms of microplastics: fibers, fragments, sheets and spherules were detected. Microplastic concentration of stream water ranged from 0 to 0.94 items L^{-1} . The lowest average ($\pm SD$) microplastic content was recorded in the pristine area (0.03 ± 0.01 items L^{-1}) followed by the rural (0.19 ± 0.06 items L^{-1}), semi-urban (0.25 ± 0.07 items L^{-1}) and the highest values were recorded in the urban area (0.56 ± 0.18 items L^{-1}) (Figure 3).

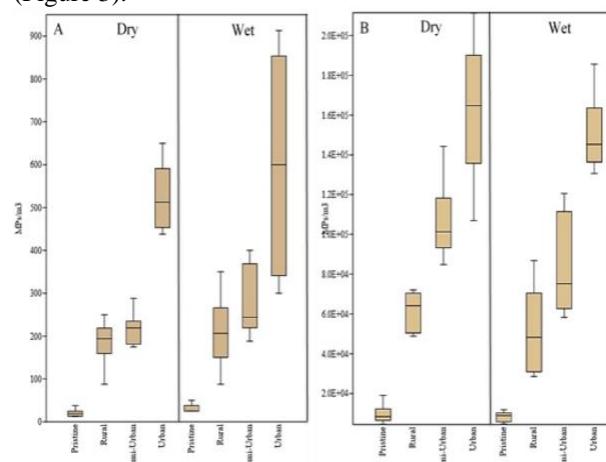


Fig. 3: Variation of microplastic content in A) water and B) sediment samples (items per m^3) in different sites along the Rawan-Oya Tributary of Mahaweli River during dry and wet seasons

According to the GLM, there were significant differences in the microplastic concentration in water samples in sites with different levels of human activity (GLM, $p < 0.0001$). A pattern similar to stream water was observed for microplastics in sediment ($n = 64$). Thus, sediment from pristine, rural, semi-urban and urban areas contained $9.07 (\pm 3.56)$, $56.14 (\pm 16.77)$, $95.1 (\pm 23.4)$, and $156.87 (\pm 26.46)$ items kg^{-1} of microplastics, respectively. According to the

FTIR analysis, out of the 13 types of polymers identified, low density polyethylene (LDPE) ($n = 100, 15\%$), polyester ($n = 81, 12\%$), polystyrene ($n = 78, 12\%$) and polyurethane ($n = 75, 11\%$) were the most numerous in water. In sediment, the most abundant polymer types were, polystyrene ($n = 217, 11\%$), resin dispersion ($n = 213, 11\%$) and polypropylene ($n = 209, 11\%$).

IV. CONCLUSIONS

This study shows that the land use patterns, anthropogenic activities and natural phenomena such as rainfall and surface runoff affect the physicochemical and microbial water quality, fish fauna and the microplastic contamination of the Rawan-Oya Tributary. The pristine area of the tributary, where human activities are minimal or absent recorded the optimum physicochemical and microbial water quality condition and lowest microplastic contamination. Only native fish species, which preferred high DO levels were recorded in this site. Urban areas had the poorest physicochemical and microbial water quality levels and the highest microplastic contamination. All tested water samples collected from urban sites were positive for human faecal contamination. A significant correlation between water quality parameters and different land use patterns was identified. Therefore, it is important that the local government propose mitigation measures to minimise these impacts in the tributary watershed area to adopt river restoration as a priority. While each is unique, all rivers face several common issues, and all can benefit from such mitigation measures.

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DEVELOPMENT OF COMPREHENSIVE INTEGRATED SOLID WASTE MANAGEMENT PLAN (CISWMP) FOR VAVUNIYA DISTRICT

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I. INTRODUCTION

Vavuniya is a fast-developing district that connects the principal towns of Sri Lanka. The inadequate qualitative and quantitative information concerning waste generation, composition, disposal, recycling, and recovery levels causes short- and long-term planning difficulties. There are no comprehensive plans for Solid waste management (SWM) at the district level, which causes difficulty in securing funds from agencies. The SWM information, appropriate strategies, and integrated SWM approaches are essential to improving SWM services and reducing greenhouse gas (GHG) emissions and environmental issues.

The Comprehensive Integrated SWM Plan (CISWMP), including institutional and infrastructure development, training programs and research, improved environmental management, resource allocation, auditing, risk and disaster management, and regulatory and law enforcement, will help determine present and future waste management needs, set priorities, and allocating resources.

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This proposed study aims to develop a Comprehensive Integrated Solid Waste Management Plan (CISWMP) based on Evidenced-Based SWM information for sustainable SWM. As such, the objectives of this proposed research were:

- i. Develop an Evidenced Based Informative and Interactive Data site (EBIIDs) based on the current information and community response regarding the SWM.
- ii. Development of strategies to reduce GHG emission reduction from the SWM sector.
- iii. Development of an SWM centre at each Local Authority (LA) level to improve the efficiency of the SWM system.
- iv. Develop a CISWMP for Vavuniya district.

II. METHODOLOGY

A. Development of EBIIDs

The semi-structured questionnaire survey was designed to collect comprehensive SWM information. The inspections were conducted to verify and assess the information and data on the SWM infrastructure and operational facilities.

Google site is a free website-building platform from Google that was customized based on the needs of developing the EBIIDs website. The website feedback system was enabled to collect responses from public and private users to enhance the available information in the future. A basic policy was drafted to update SWM information and enhance the feedback mechanism. The responsibilities are clearly defined to improve the services where necessary.

B. Estimation of GHG emission

The IPCC Methodology, considering Tier 1 estimation values based on default activity data, was used to estimate the CH₄ emission from solid waste disposal sites. According to IPCC recommendations, it was used to arrive at the expected total GHG emissions from the combustion of fossil fuels from solid waste collection vehicles.

C. Development of Waste Management Centre (WMC) and CISWMP

First, the preliminary SWM information and waste flow studies were conducted using a desk study, site visits, and key informant interviews with LA officials and stakeholders to identify the SWM issues from social, economic, and environmental perspectives. The need to revise the waste flow was clearly discussed to improve the efficiency of the SWM system. Second, the alternative technical system was finalized based on environmental economic feasibility. Then, the WMC was conceptualized, and the operation and maintenance guidelines were prepared for the successful implementation of the WMC.

The general method of work proposed for the development of CISWMP includes inception and background review, data or information capturing and analysis of gaps, framing strategies, and developing a comprehensive SWM plan.

III. RESULTS AND DISCUSSION

The total generation is 75 tons day⁻¹, and only 14 tons day⁻¹ is collected, thus having an average collection efficacy of 15%. The variations in collection efficacy are from 0 to 43%. VUC has resumed composting by processing 45% of collected waste, and only 0.1% of non-biodegradables are recycled. It is an ongoing process to gather missing data and information to promote and implement 3R. The website is functioning as a compulsive disclosure of information on SWM [<https://sites.google.com/view/swm-vavuniya-ebiid>]. Outcomes include developing the website, uploading technical reports, tabulations of data, and issues and recommendations concerning all aspects of SWM.

The Pampaimadu dumpsite, which is the largest, was identified as the main emission source. Open dumping generated 29.217Gg of CO₂ equivalent up to 2023, projected to rise by 37.8% by 2040. There will be a 57% decrease in open dumping-related GHG emissions by 2040 if composting is made mandatory for biodegradable waste, even though it produces emissions. It is much more lucrative to move towards renewable energy far sooner than planned since paying towards GHG emissions later than now will increase the commitment, which stands at 0.13 tons per person. It is derived from the deduced carbon emissions of 26,424 tons of CO₂ from the population of 203,663 of the districts in 2021. The responsibilities of the LAs will be more; thus, they need adequate support from the Central Government to implement emission reduction strategic plan.

The Waste Management Centre has been planned to be established at the LA level, which entails Material Recovery Facilities (MRF), windrow composting, and a centralized landfill with environmental protection facilities. MRF will separate the recyclables based on market needs, degradable waste will be sent to the composting facility, and a centralized landfill will be used for residual waste disposal. The material flow considered during the design stages could contribute to reducing environmental impacts.

The development of institutional and infrastructure facilities will enhance the resource allocation for SWM. Research development can identify the gaps and explain the compositional changes, which will help choose the appropriate SWM techniques for recycling, recovery, and disposal. Social development can increase stakeholder participation, where monitoring and risk assessment are vital for the SWM system's well-being. The financial mechanism is essential to enable the plans where the environmental management reduces the environmental impacts and increases the system's efficiency. Law enforcement can be helpful in successfully implementing the plan, while the CISWMP will ensure the overall sustainability of the system.

IV. CONCLUSIONS

EBIIDs will act as a tool to provide continuous comprehensive solid waste information, which helps identify the compositional changes and choose the appropriate solid waste recycling, recovery, treatment, and disposal techniques. It provides the decision-making options for the waste generators and LAs, bringing about cohesive understanding between them and other stakeholders.

Waste generations and management are essential parameters as goods and services reach the sustainable goals in Paris COP 25. As per the need, strategies were recommended to reduce the GHG emission from the Vavuniya SWM sector, such as composting degradable wastes instead of open dumping, introducing electric vehicles, and CSRP by 2025 to neutralize the cumulative GHG emission by 2040.

The establishment of WMCs has been proposed at each LA level to reduce the environmental impacts and reduce the solid waste disposal. In order to increase the efficiency of WMC, operation and maintenance guidelines are prepared to protect equipment and infrastructure facilities. The CISWMP provides the overall picture of the expected improved SWM system. CISWMP is essential to allocate the resources and prevent improper SWM practices, eventually ensuring environmental safety and economic and social feasibility.

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MICROPLASTICS BOUND TRANSPORT OF PHARMACEUTICALS, PERSONAL CARE PRODUCTS (PPCPS) AND TOXIC TRACE METALS IN WATER

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I. INTRODUCTION

Due to high levels of industrial pollution, microplastics are ubiquitously distributed in the environment and exhibit a significant affinity for toxic contaminants (Atugoda et al., 2021). Microplastics can significantly enhance the migration and dispersal of contaminants in water by acting as vehicles for them, primarily due to their hydrophobic nature and high surface area-to-volume ratio (Atugoda et al., 2020). The rate of microplastic-bound contaminant transport in water may be accelerated by the co-existence of other toxic contaminants within water systems. Furthermore, the extent of a contaminant's ability to adsorb to microplastics depends on

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factors specific to each compound, the polymeric makeup of the microplastics, and matrix effects such as salinity, pH, and dissolved organic matter (DOM) (Atugoda et al., 2021). These factors can either accelerate or impede the adsorption process, resulting in positive or negative effects on the binding mechanism.

Microplastics are known to act as vectors for pharmaceuticals, personal care products (PPCPs), and heavy metals in aquatic environments. Although adsorption data on lead ions (Pb), chromium ions (Cr), caffeine (CFN), and ciprofloxacin (CPX) exist individually, there is a lack of information regarding the sorption performance of polyethylene (PE) microplastics concerning their aging and behavior under different sorption media. Accordingly, the present study aims to generate new knowledge on the differential bio-accessibility of pristine and aged PE microplastic-bound Pb, Cr, CFN, and CPX under different sorption media. Understanding these interactions is critical for evaluating the environmental fate of contaminants and the potential risks they pose to human and ecological health.

The main objectives of the current study are to investigate the transport potential of Pb, Cr, CFN, and CPX and the influential conditions for microplastic-bound contaminants in aqueous media. To achieve these aims, the study was conducted with the following specific objectives:

- i. To determine the binding and releasing capacities of Pb, Cr, CFN, and CPX to both pristine and aged PE microplastics under varying environmental conditions such as pH, dissolved organic matter (DOM), and ionic strength of the sorption media.
- ii. To evaluate the solubility of PE microplastic-bound Pb, Cr, CFN, and CPX in gut fluid using in vitro gut fluid solubilization assays and assess the risks associated with PE microplastics as vectors based on bioavailability.

II. METHODOLOGY

A. Microplastic Preparation

Pristine polyethylene (PE) microplastics ($\sim 100 \mu\text{m}$) were purchased from MicroPowders, Inc., USA, while aged PE plastic debris ($100\text{-}250 \mu\text{m}$) was obtained by exposing PE film, purchased from the local market, to direct sunlight for one month.

B. Characterization of Microplastics

Bare and contaminant-adsorbed PE microplastics were characterized using Scanning Electron Microscopy (SEM) and Fourier Transform Infrared Spectroscopy (FTIR) analysis. The point of zero charge for both pristine and aged PE microplastics was estimated using a surface titration technique.

C. Adsorption Studies

The adsorption of Pb, Cr, CFN, and CPX onto pristine and aged PE microplastics and the influence of environmental variables; solution pH (2-10), ionic species (0.001-0.1 M of NaNO₃), dissolved organic matter (0.5-2.5 mg L⁻¹ of humic acid (HA)), and detergent-dissolved water (4% v/v) were comprehensively explored.

D. Kinetic and Isotherm Modeling

The potential binding mechanisms involved in the adsorption processes were evaluated by fitting the experimental data to various kinetic and isotherm models. Parameters were determined using Origin statistical software (version 8.0). The distribution coefficient ((K_d)) values for each adsorption process were calculated based on the equilibrium kinetic data.

E. Desorption Studies

Desorption of PE microplastics-bound Cr⁶⁺, Pb²⁺, CFN, and CPX was performed at three different pH levels: 3.0, 5.0, and 7.0, which simulate the pH conditions of stomach and gut fluids. After obtaining the desorption capacities, the percentage of bio-accessibility (BF) at each pH condition was calculated.

III. RESULTS AND DISCUSSION

As per the SEM data, both pristine and aged surfaces contain pores, while the aged PE microplastics appear coarser than the pristine ones due to the well-distributed pores on their surface. The pores and voids collectively facilitate adsorption and provide a broader surface area for the adsorbates (Atugoda et al., 2020). The point of zero charge (pHpzc) conditions of the solution were determined for both pristine and aged PE microplastics, with values of pH 4.42 and pH 3.82, respectively. Accordingly, under experimental conditions above pH 4.42, the surface of both pristine and aged microplastics is expected to display a net negative charge, and vice versa. The presence of O-containing functional groups in the FTIR spectra of aged microplastics confirmed their photooxidation.

The adsorption behaviour of Pb²⁺ and Cr⁶⁺ on both pristine and aged PE microplastics demonstrated significant variation

with the rise in pH from 2 to 8, with the maximum adsorption capacity occurring within pH 5-6 and pH 2-3, respectively. Above pH 6, Pb(OH)₂ precipitation occurs, while Pb primarily exists as Pb²⁺ in solution. The negative surface charge of PE microplastics above pH 4 facilitates electrostatic interactions with positively charged Pb ions, leading to increased adsorption. At pH values below 4.42, surface complexation between Pb²⁺ and PE microplastics contributes to adsorption. Electrostatic repulsive forces between Pb²⁺ and the PE surface hinder adsorption in the absence of NaNO₃ and humic acid (HA), emphasizing the role of electrostatic interactions in the sorption process. At lower pH levels, Cr⁶⁺ ions exist primarily as oxyanions like HCrO₄⁻, CrO₄²⁻, and Cr₂O₇²⁻, with HCrO₄⁻ being predominant. The positively charged surface of microplastics at pH 2-3 increases affinity for HCrO₄⁻ ions via electrostatic interactions. However, beyond pH 2, decreased H⁺ ions lead to increased negative charge density on the microplastics' surfaces, reducing adsorption efficiency due to repulsive effects between HCrO₄⁻ and negatively charged surfaces. Competitive effects also occur between HCrO₄⁻ and OH⁻ ions for adsorption sites.

The best pH condition for both CFN and CPX adsorption was pH 6-7, where the microplastics' surfaces have a net negative charge. Within this pH range, CFN exists in both protonated (CFNH⁺) and deprotonated (CFN) forms, with CFNH⁺ being predominant. This allows CFNH⁺ to readily attract to the PE surfaces via electrostatic forces. At pH < 4.4, adsorption capacities were lower due to electrostatic repulsion between positively charged PE surfaces and CFNH⁺ species. Subsequently, the adsorption capacity gradually increased but dropped again with increasing pH beyond 7.5 and 6.0 for pristine and aged PE microplastics, respectively. This decrease may result from electrostatic repulsion between anionic CFN and negatively charged PE surfaces. The pH dependency of CFN adsorption highlights electrostatic forces as the primary interaction. Additionally, hydrophobic interactions between the microplastics' hydrophobic faces and neutral CFN molecules may also contribute to adsorption. Below pH 6.1, electrostatic repulsion occurs between the protonated PE surface and the cationic form of CPX (pKa₁ = 6.1). As pH increases towards 6.0, the more negative surfaces of the microplastics enhance electrostatic forces, leading to increased CPX adsorption. Between pH 6.1 (pKa₁) and 8.7 (pKa₂), CPX exists mainly in the zwitterionic form (CPX^{-/+}), with both CPXH₂⁺ and CPX^{-/+} forms present within the optimal pH range. Electrostatic interactions may occur between the negatively charged microplastic surfaces and protonated CPX, while hydrophobic interactions could involve the dominant zwitterionic forms and hydrophobic microplastic surfaces. However, above pH 7.5, electrostatic repulsive forces between negatively charged CPX forms and microplastics result in a significant decrease in adsorption capacity. This suggests that microplastics could potentially serve as carriers for CPX transfer through natural water, particularly in circum-neutral environments like fresh surface waters.

The best sorption medium for Cr⁶⁺ adsorption (0.65-0.80 mg g⁻¹) was detergent-dissolved water, whereas it was HA-mixed water for Pb²⁺ (1.05-1.34 mg g⁻¹), CPX (1.59-2.22 mg g⁻¹), and CFN (1.27-2.25 mg g⁻¹) adsorption. Increasing HA concentration from 0.5 to 2.5 mg L⁻¹ enhanced the binding capacity of PE microplastics for CFN, CPX, and Pb²⁺ adsorption, whereas increasing NaNO₃ concentration

gradually declined the adsorption. Adding more ions to the sorption medium affects the adsorption of Pb^{2+} onto PE microplastics. Cations like Na^+ , Mg^{2+} , Ca^{2+} , and K^+ compete for binding sites on the microplastic surface, reducing Pb^{2+} adsorption due to electrostatic interactions (Atugoda et al., 2021). Higher ionic strengths decrease sorption capacity by altering the PE surface charge and promoting agglomeration, which hinders metal adsorption. In the presence of humic acid (HA) at pH 5-6, deprotonated carboxylic and phenolic groups attract Pb^{2+} via electrostatic interactions, while hydrophobic regions of HA enhance microplastic surface hydrophobicity.

However, excessive organic matter inhibits Pb^{2+} adsorption through complexation interactions. The interplay between ion competition, surface charge, and organic matter content significantly influences Pb^{2+} adsorption onto PE microplastics. The initial batch sorption experiments with Cr^{6+} using $NaNO_3$ and HA showed inconsistent and low adsorption. Switching to detergent-dissolved water increased Cr^{6+} adsorption on both pristine and aged PE microplastics. Anionic surfactants in the detergent likely contributed to this. At pH 2.0-3.0, detergent-dissolved water significantly enhanced Cr^{6+} adsorption. The high surface area-to-volume ratio of microplastics, along with their hydrophobic nature, allows them to aggregate on the water's surface. When anionic surfactant molecules are present in detergent, they can enhance the adhesion and mobility of microplastics through hydrophobic interactions. This lowers the surface tension of the water, stabilizing the dispersion of microplastics and increasing the available surface area for Cr^{6+} adsorption. In low pH environments, the anionic head of the surfactants can interact with H^+ ions, facilitating interactions with Cr oxyanions (Zhang et al., 2020). Cr^{6+} can then bind to the microplastic surface via surface complexation. However, at high pH, the decrease in H^+ ions and increased electrostatic repulsive forces can reduce Cr^{6+} adsorption. Anionic surfactants can bind both Cr^{6+} ions and microplastics, affecting dispersion in the sorption medium. Overall, the adsorption mechanism primarily relies on electrostatic and hydrophobic interactions, with surfactants playing a crucial role as either hydrophobic attractors or positive charge interactors.

The changes in CFN and CPX adsorption might be caused by the increase in viscosity and density of the sorption medium with the introduction of ionic species. This can hinder the movement of molecules, reducing their mobility toward the microplastics. Thus, since ionic species can more easily access the binding sites on the microplastic surface compared to molecular CFN species, Na^+ and NO_3^- can compete with CFN molecules for the available adsorption sites on PE microplastics. PE microplastics develop a net negative surface charge at the optimal pH for CFN and CPX adsorption, enabling them to attract positively charged moieties through electrostatic interactions. Carboxylic and phenolic groups in HA protonate with H^+ ions in the medium, forming positively charged groups, which bind to the negatively charged PE microplastic surfaces electrostatically. Additionally, hydrophobic regions in HA molecules interact with hydrophobic PE surfaces, indicating that both hydrophobic and electrostatic forces govern the binding of HA to PE microplastics. The increased adsorption capacity of PE microplastics with higher HA concentrations suggests that HA promotes the adsorption process. Moreover, HA can rearrange its conformation and disrupt intramolecular H-bonds,

facilitating beneficial intermolecular H-bonding interactions with PPCPs. The interaction between CFN/CPX and HA involves several favorable reactions: hydrogen bonding between their atoms, nucleophilic interactions, and $\pi-\pi$ stacking interactions. However, strong $\pi-\pi$ stacking can lead to steric destabilization. Van der Waals interactions may also contribute to the binding mechanism between CFN/CPX and organically modified PE surfaces. CFN and CPX can also interact with free HA molecules in the water, potentially reducing their mobility and adsorption probability by PE microplastics. CFN and CPX can directly interact with PE microplastics without HA involvement. Protonated CFN can bind to negatively charged sites on microplastics, while uncharged or anionic forms can utilize hydrophobic interactions for binding, as both PPCPs and HA promote electrostatic attraction to microplastics. In summary, ionic species, especially anionic surfactants in detergent solutions, significantly influence the adsorption of PPCPs onto PE microplastics through complex interactions. While the effect of ionic strength on microplastics remains unclear, surfactant presence alters microplastic dispersal and aggregation behavior, resulting in varying adsorption efficiencies.

IV. CONCLUSIONS

This study assessed the adsorption and desorption performances of pristine and aged PE microplastics for Pb^{2+} , Cr^{6+} , CFN, and CPX. The highest retention of Pb^{2+} , Cr^{6+} , CFN, and CPX by aged microplastics was attributed to their abundant oxygen-containing groups. Solution pH was another key factor affecting the adsorption/desorption characteristics of microplastics. The adsorption capacities of PE microplastics for Pb^{2+} , CFN, and CPX in water were predominantly influenced by an increase in HA concentration under varying organic matter saturation around their surfaces. Surfactants in detergent-dissolved water significantly contributed to the enhanced adsorption of Cr^{6+} . The adsorption of Pb^{2+} and Cr^{6+} ions by microplastics was largely driven by electrostatic and hydrophobic interactions, whereas CFN and CPX adsorption were controlled by van der Waals forces, intermolecular hydrogen bonds, or nucleophilic attractions. Primarily, PE microplastics exhibited rate-limiting chemisorption and physisorption or cooperative adsorption for Pb^{2+} , Cr^{6+} , CPX, and CFN with increasing contact time and adsorbate concentration, respectively. The higher bioaccessibility fractions reported for Pb^{2+} and CPX in simulated gastric fluid, and for Cr^{6+} in simulated gut fluid, indicated the possibility of releasing microplastics-bound Pb^{2+} , Cr^{6+} , and CPX in human body fluids after bioaccumulation. Lastly, the findings of this study suggested that detergent-dissolved water enhances the vector transportation of Cr^{6+} , whereas HA-mixed water promotes the vector transport of Pb^{2+} , CPX, and CFN through pristine and aged PE microplastics.

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INSTITUTION WHERE RESEARCH WAS CARRIED OUT

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CONSERVATION STATUS AND AN ASSESSMENT OF THE CROP DAMAGE CAUSED BY THE HOG DEER *Axis porcinus* (ZIMMERMANN, 1780) IN GALLE DISTRICT SOUTHWESTERN SRI LANKA

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I. INTRODUCTION

Hog deer (*Axis porcinus*) is the most endangered member of family Cervidae and the rarest species in Sri Lanka (Yapa and Ratnavira, 2013). Sri Lanka together with the Western Ghats is one of the 36 biodiversity hotspots with a highly diverse endemic fauna that is facing a high level of threats (Myers et al., 2000; Habel et al., 2019). Sri Lanka has four deer species namely Spotted deer (*Axis axis*), Sambar deer (*Rusa unicolor*), Barking deer (*Muntiacus muntjak*), and Hog deer (Padmalal et al., 2003). Of them, Hog deer is the most elusive and shy member that shows an awkward distribution (McCarthy and Dissanayake, 1994).

Although several studies have been conducted on other deer species, a little work have been carried out on Hog deer in Sri Lanka. This may be due to its elusive, nocturnal habits, and limited distribution. A little is known about their ecology in Sri Lanka. Therefore, it is necessary to investigate the present conservation status and threats of Hog deer.

The main objectives of this study were to find the conservation status and the damage incurred by Hog deer to cinnamon plantations in Galle district South-western Sri Lanka.

To achieve the aims, study was conducted with the following specific objectives:

- i. Assess the present distribution range of Hog deer
- ii. Assess the damage caused by Hog deer to the cinnamon plantations
- iii. Assess the threats to Hog deer in its range of distribution
- iv. Assess the perceptions of farmers toward the Hog deer
- v. Propose protective measures and conservation initiatives.

II. METHODOLOGY

The study area is located between Bentota and Gin Rivers in Galle District. The area supports riverine mangroves and marshes associated with rivers. Further, the Galle District has one of the highest human population densities in Sri Lanka (DCS, 2018). Several commercial crops including cinnamon (*Cinnamomum verum*) being the major, Coconut (*Cocos nucifera*), paddy (*Oryza sativa*), tea (*Camellia sinensis*), oil palm (*Elaeis guineensis*), and rubber (*Hevea brasiliensis*), are found.

A rough idea of the Hog deer distribution in the Galle District has been taken from the information compiled at the Hiyare Biodiversity, Education and Research Centre (HBERC). This facility receives disabled and injured Hog deer in and around Galle district.

The questionnaire survey was conducted within the study area to assess the crop damage, peoples' perceptions, and threats to Hog deer, etc. Randomly selected Grama Niladari Divisions (GND) in the Galle District were visited covering approximately 75% of the area. One person was involved in interviewing the people including Buddhist monks, farmers, landowners, plantation workers, fishermen, poachers, caretakers (plantations), boatmen, and sand miners. Later, a structured questionnaire form containing fifteen open-ended questions which were made using a standard interview format and photographic identification cards of the four Sri Lankan deer species were used (Arshad et al., 2012). The feedback from local communities was used to compile information on the presence or absence of the species in a particular area (Arshad et al., 2012).

The transects were conducted in randomly selected cinnamon cultivation. A total of 1,376 (229.5 ± 34.9) cinnamon bushes have been counted during the transect survey.

The indirect signs of their presence including crop damage, footprints, dung piles, nesting sites, etc. were collected during the field surveys. *Muntjac* tracks can be identified by having consistent tracks with one cleave longer than the other (Brown et al., 1992). and the *Axis* deer tracks usually have slightly elongated slots parallel including dew claws are absent, and the bigger tracks on the damp ground showing slight slide forward with no spay (Brown et al., 1992). The wild boar track is generally larger in size and it often holds together even when dry (Brown et al., 1992). Further, the domestic goat tracks are small with curved and round ends that are distinguishable from deer tracks [8], and the goats are not present in the study area. The wild boar dropping can be distinguishable by having dark feces and resembling half-inflated rugby balls (Brown et al., 1992). The *Muntjac* pellets are black and shiny with rounded facets, striation and a single peak small about 1 cm in diameter sometimes as an intact mass (crottie), more often widespread (Brown et al., 1992).

Further, the qualitative threats assessment method was conducted to assess the level of threats that Hog deer faces. The qualitative threats assessment method was used based on the guidelines given in Standards Australia (2009). The level of threats was determined by the likelihood and consequence of the threat to the species. Vulnerability to threats was inferred by biological variables known to affect extinction risks such as population size, population decline, geographic ranges, and the number of extant populations (O'Grady et al., 2004; Mace et al., 2008).

III. RESULTS AND DISCUSSION

A few direct sightings were recorded indicating Hog deer is an elusive and shy animal. A total of forty-one individuals were recorded from Kosgoda, Uragaha, Karandeniya, Elpitiya, Batapola, Hikkaduwa, Gonapinuwala, and Geoffrey Bawa Estate. A total of 27 (1.3 ± 1.7) disabled and injured Hog deer including three fawns were received to HBERC during the period from July 2020 to February 2022 for one and a half years.

Although direct sightings were minimal, their indirect signs were rather common in the study area including footprints, droppings, nesting sites, and crop damage. Indirect records were common, especially in the following areas such as Kosgoda,

Uragaha, Karandeniya, Elpitiya, Batapola, Hikkaduwa, and Gonapinuwala. A total of 246 questionnaire forms were filled by over 500 people from the study area. In the case of group of people, only one form was filled. Of the recorded 246 questionnaires, 175 (71.13%) reported that Hog deer were present in their vicinity. These places have also been confirmed from their indirect records. Of the 246 interviews, 17 (6.91%) reported that Hog deer were not common in their

vicinity. Fifty-four (21.9%) have reported that they were absent in their vicinity. Based on the questionnaire, people have seen a total of 306 (1.24 ± 2.2) adults and 22 (0.08 ± 0.37) fawns from the study area that are from the most recent times.

Deer damage cinnamon by eating new shoots after the harvest and bark gets damaged due to antler rubbing during the rutting season. Moreover, they damage paddy due to trampling and sometimes eat young rice plants. Further, they make nesting sites in the paddy fields during the harvesting time. They also damage the vegetables especially the green leaves. The highest damage occurred to cinnamon (50%), followed by paddy (25%), and vegetables (6%).

A total of 112 (45.52%) responded that electrocution was the problem for Hog deer followed by shooting 59 (23.98%) of respondents. The least reported threat for the Hog Deer was falling into wells (0.41%). Road accidents, dog attacks, and setting snares for deer are also at a high level. People reported water monitor attacks, falling into wells, habitat destruction, keeping as a pet, and "Hakka Patas" as threats. The Hakka Patas (a kind of homemade small explosive device) was a new method of killing animals in the area. Other threats include fire crackers, pesticides, pitfall traps, trap guns and attacks by other animals (Python, *Python molurus*).

Only 49 (8.17 ± 7.73) sapling damages which are about 163.3 trees/hectare and only nine (1.5 ± 2.74) antler rubbings which are about 30 trees/hectare have been recorded. Hence, these results obviously indicated that the damage to the cinnamon plants was not at a higher level. Most of the time these damages occurred to a part of the plant but not to the entire plant.

A total of 175 confirmed Hog deer locations have been identified from this survey. Their distribution lies in the Galle District bordering to Bentota River from the north, the Gin River from the south, a one to two-kilometer inland coastal line from the west and the southern highway from the east.

As per the qualitative threat assessments impact on the population, fragmentation of habitat, impact on the habitat critical to survival, and human pressures are also at major and critical levels.

IV. CONCLUSIONS

Hog deer are restricted to land area of less than 250 square kilometers in the Galle District in the southwestern part of Sri Lanka. Small aggregations can be observed in areas including Kosgoda, Uragaha, Karandeniya, Elpitiya, Batapola, Hikkaduwa, and Gonapinuwala. Hog deer abundance was low within the area hence direct records were minimum. However, their indirect records were rather common within the study area.

The major crop damaged was cinnamon followed by paddy and vegetables. These damages were very low and only happened in areas where Hog deer were particularly common. The damage was not constant and changes with the

season and the availability. Hence the damage was tolerable for the people and people's perception was not critical.

Nevertheless, Hog deer are facing enormous threats in the area. The major threats are electrocution, shooting, dog attacks, and road accidents. The threats that the Hog deer face are critical in the study area since laws to protect animals outside the protected areas are also minimum. Sometimes, people claim that Hog deer should be protected and have the right to live as humans.

Therefore, proper conservation plans with sound documentation participation with all the stakeholders (government, private, researchers, etc.) are necessary for future management of Hog deer in Sri Lanka.

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INSTITUTION WHERE RESEARCH WAS CARRIED OUT

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APPLICATION OF FOUR BODY CONDITION SCORING INDICES TO ASSESS THE WELL-BEING OF ASIAN ELEPHANT (*Elephas maximus maximus*) AT THE PINNAWALA ELEPHANT ORPHANAGE, SRI LANKA

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I. INTRODUCTION

The Sri Lankan elephant (*Elephas maximus maximus*) had been declared endangered in 2008 (Rathnayake et al., 2022; Sukumar, 2006). The primary threat to elephants in Sri Lanka, as elsewhere, is habitat loss and fragmentation, leading to Human-Elephant Conflict (HEC). Annually, due to HEC, 70 humans and 250 elephants lose their lives (Fernando et al., 2011), and about 14 elephant calves become orphans (Perera et al., 2018). Pinnawala Elephant Orphanage (PEO) is an institution for taking care of orphaned elephants in the wild in Sri Lanka. Currently, PEO is caring for 74 elephants, including 33 males and 41 females (DNZG, 2022). Each elephant has its own individual name for identification. As a decent indicator of health and management, the body condition score (BCS) index is being subjectively used, which measures the body fat reserves of animals, particularly over the bony prominences. Recently, researchers have developed several BCS indices to assess the body conditions of elephants. Four widely used BCS indices, namely (Wemmer et al., 2006; Fernando et al., 2009; Wijeyamohan et al., 2014; Morfeld et al., 2016) are internationally documented for Asian elephants.

The study aimed to assess the body condition of elephants in the PEO using BCS indices, to determine the most suitable BCS index. Additionally, efforts were made to identify

outlier elephants and propose remedies to enhance veterinary attention and improve their respective situations.

II. METHODOLOGY

The study exclusively focused on 62 elephants, 27 were males (mean age: 19.33 years; age range: 9–66 years), while the remaining were females (n=35; mean age: 27.66 years, age range: 10–57 years).

The body condition scoring indices developed by [6] (BCS_{We}), [7] (BCS_{Fe}), [8] (BCS_{Wi}) and [9] were used separately to assess the body condition of selected elephants.

After assigning the respective BCS using all four methods, their relationships with gender, age, and origin were examined using nonparametric statistical tests. The correlation among the four different BCS indices was calculated using the Spearman rank correlation coefficient statistical test at 5% significance. The elephants studied were separated into 5 age groups (9-19, 20-29, 30-39, 40-49, 50≤) and average $\pm SD$, median, first quartile and third quartile for four BCS indices were compared using a nonparametric statistical test. A nonparametric two-sample test (Wilcoxon rank sum test) was conducted to compare the median BCS index between the two sexes. A nonparametric two-sample test (Wilcoxon rank sum test) was performed to examine whether the median BCS index estimated using four methods differs according to the origin of the elephant. Boxplots were drawn for each BCS indices to determine whether any outliers exist.

III. RESULTS AND DISCUSSION

All pairwise comparisons between the four methods of BCS calculations had a significant positive linear relationship ($r > 0.75$ with $p\text{-value} < 0.052$). Hence, all BCS calculation systems appear to give similar biological results and all can be used to examine BCS in captive Asian elephants.

No significant relationship was identified between the age of the elephant and the method of BCS index calculation, which may be primarily due to the presence of only four (8.06%) elephants above 50 years of age in PEO. The comparison of males and females on BCS calculated using four methods did not show any difference between the two, which means males and females do not differ in their body

condition in PEO. Comparisons between captive-born and wild-origin elephants revealed no significant differences in body condition across all four methods of BCS estimation. The majority of wild elephants brought in as smaller calves below 1 year old have now grown into at least sub-adults or older elephants, showing that with adaptation, their body condition becomes similar to those born in PEO.

The BCS estimation with all four methods is not normally distributed and the individuals with extreme scores can be identified for each BCS index. According to the boxplots, Neela (48 years old) had the lowest body scores across all BCS calculation methods. Additionally, Neela, along with Ninja (36 years old), Thammanni (33 years old), and Kumari (57 years old), were identified as outliers on the lower end, indicating relatively weaker body condition.

In PEO, veterinarians periodically adjust the food ration considering the availability of the plant varieties that elephants feed; hence, regardless of the season, elephants at the PEO receive a daily provision of all the necessary food for their nutrition. In addition, in PEO, elephants are provided with all the resources they require, eliminating any competition. Therefore, other factors such as health, age, and genetic defects may have contributed to these elephants becoming outliers.

The body condition of elephants deteriorates to some extent when they get old. Considering the outliers, Kumari is the only one over 50 years of age, and due to its age, it has been an outlier. Considering other outliers; Neela, Tammani and Ninja, it can be decided that health-related senescence, tuberculosis, arthritis and growth defects have contributed to their poor body conditions, resulting in them being outliers.

Six elephants have taken full scores for all BCS indices, and they may be obese or overweight. All of them are females and are middle-aged. Except for one, the other five were mothers. The reason for this obesity may be their middle age, which may result in high-fat deposition and motherhood. However, no male elephant could achieve a full score for all BCS indices. Captive adult males undergo a long musth period, lasting from two to nine months at PEO. During musth, males typically have a reduced appetite, which may explain why no male received a full score. Considering the young elephants below 20 years old, none have been able to achieve a full score may be attributed to their high activity levels and relatively faster metabolism at this age. Consequently, their fat deposition may be lower compared to middle-aged elephants.

IV. CONCLUSIONS

Food and nutrition, and environmental factors such as climate, weather patterns, and natural disasters, which typically influence the body condition of elephants in their natural habitat, do not exert a significant influence on the body condition of elephants within the PEO. Accordingly, hereditary diseases, prolonged health ailments, and age can be concluded as the primary factors influencing the body condition of elephants within the PEO. Consequently, it can

be inferred that the overall body condition of the elephant population at PEO is satisfactory.

All BCS indices that were used in the study, BCS_{We} , BCS_{Fe} , BCS_{Wi} and BCS_{Mo} are useful in studying the body condition of an elephant. However, BCS_{Fe} is more useful and reliable in analyzing the body condition of elephants in captive elephant management centres. So, BCS_{Fe} is the ideal index for scoring body condition for PEO.

Pinnawala Elephant Orphanage cares for Sri Lanka's largest population of captive elephants. As an institution that cares for such a large captive population, the use of BCS is critical to detect changes in elephant body condition easily. Body condition scoring indices for elephants are easy to use and can be easily worked with by curatorial staff. It is recommended to use the BCS_{Fe} once a quarter to assess elephant body condition in PEO, as it is easy to use and easily understood by curatorial staff.

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SOIL AND GEOCHEMICAL CHARACTERISTICS OF ELEPHANT GEOPHAGIC SOILS IN UDAWALawe NATIONAL PARK, SRI LANKA

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I. INTRODUCTION

Geophagy, or soil consumption, from natural licks is reported in many mammalian herbivore and omnivore populations (Klaus *et al.*, 1998). Numerous mammals and birds visit mineral licks to ingest soil or drink water. These mineral licks are most commonly found along riverbanks but are also present within forests. Geophagy has multiple causes that may vary geographically, seasonally, and taxonomically, resulting in temporal and spatial variation in the use of mineral licks. The proposed benefits of geophagy include mineral supplementation, detoxification of plant secondary compounds, and alleviation of digestive disorders. Changes in diet often influence the need for geophagy, and reproductive activity stages can also affect the usage of mineral licks. For example, the concentration of sodium in mineral licks may vary across different locations (Blake *et al.*, 2011).

In Sri Lanka, there remains limited scientific evidence addressing elephant geophagic locations within the country. To date, only one research article has examined the characteristics of elephant geophagic soil in Udawalawe National Park, conducted by Chandrajith *et al.* in 2009. This study represents the only scientific evidence available on this

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subject in Sri Lanka. Their findings indicated no significant geochemical differences between geophagic and non-geophagic soil groups in Udawalawe National Park, with the only notable distinction being in the clay mineralogical content. However, research findings from other countries have revealed that geophagic soil contains significantly higher quantities of sodium, potassium, calcium, magnesium, phosphorus, manganese, and clay compared to other soils. Consequently, further research is needed to investigate the geochemical and geophysical characteristics of elephant geophagic soil in Udawalawe National Park.

The general objective of this research was to investigate the potential reasons for elephant geophagy in Udawalawe National Park (UWNP).

The specific objectives of this study were as follows:

- i. To identify the differences of physical properties in geophagic and non-geophagic soil.
- ii. To identify the differences of selected chemical properties in two soil groups.
- iii. To identify the differences of the clay mineralogical content in these soil groups.

II. METHODOLOGY

The study area was Udawalawe National Park (UWNP), with the majority of the park situated in the intermediate climatic zone, while a portion lies within the dry zone. In this research, seven elephant geophagic locations were identified for sampling (Fig. 1). Geophagy was observed in the topsoil, extending to a depth of approximately 30 cm in the soil licks. Three random samples were collected from each geophagic location down to the level created by elephant geophagy. A representative sample for each location was created by mixing the three collected samples. Additionally, five non-geophagic locations in close proximity to the geophagic sites were sampled in the same manner for comparative analysis.



Fig. 1: Elephant Geophagy of Soil in Udawalawe National Park (Picture Source- Mobile phone capturing by Wild Life Ranger Mr D.A.J Anil Vithanakanda)

All composite soil samples were stored separately in polythene bags and air-dried.

All soil samples from the two soil groups underwent particle size separation using the pipette method (Gee and Or, 2002). The pH of the soil samples was then measured using a pH meter in a soil suspension of 25 ml KCl (soil-to-solution ratio of 1:2.5), as described by McLean *et al.*, 1982.

A soil-to-solution ratio of 1:5 was used to analyze the soil's electrical conductivity (EC) using an EC meter for each sample. Subsequently, all soil samples were analyzed to determine total soil phosphorus, nitrogen, potassium, calcium, and magnesium content.

Total soil phosphorus was determined using the sodium hypobromite/sodium hydroxide alkaline oxidation method (Dick and Tabatabai, 1977). Total soil nitrogen was analyzed using the Kjeldahl procedure (Liao, 1981). To determine total sodium, potassium, calcium, and magnesium, a 2.5 g sample of air-dried soil was placed into a 125 ml Erlenmeyer flask, followed by the addition of 50 ml of 0.1 M hydrochloric acid as the extracting solution. The mixture was agitated for 15 minutes using an automated shaking machine, then filtered through Whatman No. 12 folded filter paper into a 50 ml Erlenmeyer flask. A standard series was prepared for each element. Total potassium was measured using a flame spectrophotometer, while total sodium, magnesium, and calcium were measured using an atomic absorption spectrophotometer (Bruppacher, 1968).

Seven samples (five geophagic and two non-geophagic) were subjected to mineralogical analysis using X-ray diffraction (XRD) to determine the presence of major and clay minerals. The analyses were conducted in the range of 5–80° 2θ, with a step size of 0.020° 2θ.

III. RESULTS AND DISCUSSION

Field observations in Udawalawe National Park (UWNP) revealed that the Hulankapolla (G1), Handagiriya near office premises (G6), and Handagiriya Kiullinda Ara (G7) elephant geophagic sites were particularly popular among elephants. The Handagiriya Kiullinda Ara (G7) geophagic site was located on a riverbank, where a hole had been created due to elephant geophagy. Wildlife officers have observed that this location was also frequented by other mammals, such as deer and wild pigs, in addition to elephants.

The determination of soil texture indicated a greater diversity of soil textural classes within the elephant geophagic soil group compared to the non-geophagic soil.

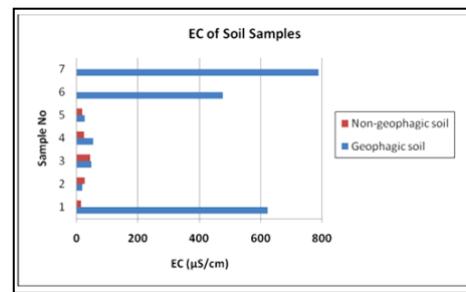


Fig. 2: EC of Geophagic and Non-geophagic Soils, $t = 0.0064$, $DF = 10$, $Gmean = 290.29$, $NGmean = 25.22$

Regarding the chemical analysis of the soil, significantly higher electrical conductivity values were observed at the most popular elephant geophagic sites: G7 ($788.45 \mu\text{Scm}^{-1}$), G1 ($623.35 \mu\text{Scm}^{-1}$) and G6 ($476.20 \mu\text{Scm}^{-1}$). These results indicate that these soils may contain elevated levels of soluble salts.

Additionally, G7 and G6 exhibited the highest pH values, recorded at 9.61 and 9.05, respectively. All geophagic soil samples showed higher concentrations of the tested trace elements, specifically total Na^+ , Ca^{2+} and Mg^{2+} ions, compared to non-geophagic soils. No significant difference in total nitrogen, phosphorus, or potassium was observed between the geophagic and non-geophagic soil groups in UWNP.

IV. CONCLUSIONS

It can be concluded that the elephant geophagic soils in Udawalawe National Park (UWNP) are extremely weathered and rich in sodium, calcium, and magnesium compared to the non-geophagic soils in the park. Therefore, both the presence of soluble salts—namely sodium, calcium, and magnesium—and the occurrence of weathered clay minerals such as kaolinite in the soil contribute to the geophagy of elephants in UWNP, allowing them to fulfill their nutrient requirements and address dietary disorders, such as detoxification.

Further research is necessary to investigate other potential reasons for elephant geophagy, including the relationship with soil parent materials, detailed behavioral studies of elephants' geophagy, and the identification and mapping of all elephant geophagic sites for the purpose of conservation within the demarcated area in UWNP.

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MATHEMATICS

A UNIFIED APPROACH TO DIFFERENCE FORMULAS FOR FRACTIONAL AND INTEGER ORDER DERIVATIVES

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I. INTRODUCTION

Grünwald approximation (GA) for fractional derivatives (FDs) is a widely used tool for numerically solving fractional differential equations (FDEs). The weights of the GA are obtained from the coefficients of the generating function $(1-z)^\alpha$ expressed in a power series form, where α is a fractional order derivative of a given function. A shifted form of the GA was proposed in the literature to remedy some stability issues in solving fractional diffusion equations. Both the GA and its shifted form are of first order approximations.

For higher order approximations, the author, Lubich, has obtained some generating functions in the form of fractional power of polynomials (Lubich, 1986). The coefficients of the expansion of the generating functions provide the coefficients for the Grunwald type approximation (GTA) with higher order accuracy without shift. Shifted forms with these coefficients give only first order approximations regardless of their higher order in non-shifted forms. Recently, higher order GTAs with shift have been obtained by Nasir and Nafa and applied to fractional diffusion equations with second order accuracy (Nasir & Nafa 2018). The coefficients for the generating functions of GTAs were often obtained by hand computations or by symbolic computations.

Most of scientific research with mathematical pattern recognition are aimed at automating the relevant scientific processes. In this regard, in numerical approximations, especially in numerical calculus, it is desirable to have general formulas for derivatives that can be automated to give desired approximation formulas.

The present authors have obtained an explicit form for the coefficients of the generating functions that gives GTAs for fractional derivatives with shifts retaining their higher orders. This form generalizes the Lubich form with shift and hence the Lubich form becomes a special case with no shift. Interestingly, this explicit form also gives coefficients for finite difference formulas (FDFs) for integer-order derivatives as demonstrated by this explicit form. However, it gives higher order compact FDFs for the first derivative only. Here, a compact FDF means the FDF which used minimum number of function values at the discrete nodal points. For higher integer-order derivatives, the resulting FDA are not compact although they are valid for FDFs.

Usually, the derivation of the weights for the FDFs for integer-order derivatives involves linear combinations of the Taylor series expansions of a function at various grid points about the point of derivative. This leads to, higher order accuracy requirements, uncontrollable hand calculations, solving systems of linear equations or heavy symbolic computations. Besides, this technique is not suitable for fractional derivatives as the latter involves function values at infinite grid points, due to its non-local nature.

Explicit forms for FDFs have appeared in the past. However, all of them are focused on the FDFs for integer-order derivatives only.

In this study, we extend the foregoing explicit form to a more general unified explicit form that gives newer GTAs for fractional derivatives and compact FDFs for any integer-order derivatives.

II. METHODOLOGY

The shifted form of GA of a left(-)/right (+) fractional derivative, $D_{x\pm}^\alpha f(x)$, $\alpha > 0$, of a function $f(x)$, with shift $r \geq 0$, from the point of the derivative is given by:

$$\delta_{\pm}^{(\alpha)} f(x) = \frac{1}{h^\alpha} \sum_{k=0}^{\infty} g_k^{(\alpha)} f(x \mp (k-r)h) \quad (1)$$

where the Grunwald weights, $g_k^{(\alpha)} = \frac{(-1)^k \Gamma(\alpha+1)}{k! \Gamma(\alpha-k+1)}$, are obtained from the coefficients of the expansion of the generating function $W_1(z) = (1-z)^\alpha$ and can be computed via the recursive formula [1], $g_0^{(\alpha)} = 1, g_k^{(\alpha)} = \left(1 - \frac{\alpha+1}{k}\right) g_{k-1}^{(\alpha)}, k = 1, 2, 3, \dots$

A generalized form of the Grünwald approximation was proposed by Nasir and Nafa [2]:

$$\Delta_{\pm h, r, p}^{(\alpha)} f(x) = \frac{1}{h^\alpha} \sum_{k=0}^{\infty} w_k^{(\alpha)} f(x \mp (k-r)h) \quad (2)$$

where the weights, $w_k^{(\alpha)}$, are to be determined for a required order of approximation p and shift r . We call (2) the *Grunwald type approximation* (GTA) and the weights $w_k^{(\alpha)}$ as *Grunwald type weights* (GTWs).

Proposition 1: Let $\alpha > 0$, $n = [\alpha] + 1, m$, a non-negative integer, a function $f(x) \in C^{m+n+1}(\mathbb{R})$ and $D^k f(x) = \frac{d^k}{dx^k} f(x) \in L_1(\mathbb{R})$ for $0 \leq k \leq m+n+1$. Then, the generating function $W(z)$ approximates the left and right fractional differential operator $D_{x\pm}^\alpha$ with order p and shift r , $1 \leq p \leq m$, if and only if

$$G(z) := \frac{1}{z^\alpha} W(e^{-z}) e^{rz} = 1 + O(z^p) \quad (3)$$

Moreover, if $G(z) = 1 + \sum_{l=0}^{\infty} a_l z^l$, where $a_l = a_l(r)$, then we have

$$\begin{aligned} \Delta_{\pm h, r, p}^{(\alpha)} f(x) &= D_{x\pm}^\alpha f(x) + h^p a_p D_{x\pm}^{\alpha+p} f(x) \\ &\quad + h^{p+1} a_{p+1} D_{x\pm}^{\alpha+p+1} f(x) + \dots \\ &\quad + O(h^p) \end{aligned} \quad (4)$$

This characterization theorem was used in Proposition 2 to obtain generating functions of the form $W(z) = (\beta_0 + \beta_1 z + \dots + \beta_p z^p)^\alpha$ for an approximation of order p with shift r .

Proposition 2 The generating functions $W_{p,r}(z) = (\beta_0 + \beta_1 z + \dots + \beta_p z^p)^\alpha$ approximates the fractional derivatives with order p and shift r if and only if

$$\beta_j = - \left(\sum_{\substack{m=0 \\ m \neq j}}^p \prod_{\substack{l=0 \\ l \neq m, j}}^p (\lambda - l) \right) \left(\prod_{\substack{m=0 \\ m \neq j}}^p \frac{1}{j-m} \right) \quad (5)$$

where $j = 0, 1, 2, \dots, p$, $\lambda = r/\alpha$, and the leading error constant is given by

$$R = \frac{1}{(p+1)!} \sum_{j=0}^p (\lambda - j)^{p+1} \beta_j \quad (6)$$

The weights $w_k^{(\alpha)}$ of $W_{p,r}(z) = (\beta_0 + \beta_1 z + \dots + \beta_p z^p)^\alpha = \sum_{k=0}^{\infty} w_k^{(\alpha)} z^k$ are computed via the recursive formula:

$$\begin{aligned} w_0^{(\alpha)} &= \beta_0^\alpha \\ w_k^{(\alpha)} &= \frac{1}{k \beta_0} \sum_{j=1}^K (j(\alpha+1) - k) w_{k-j}^{(\alpha)} \beta_j \end{aligned} \quad (7)$$

where $k = 1, 2, 3, \dots$ and $K = \min(k, p)$.

The main result of this study is presented by Theorem 1 and Theorem 2.

Theorem 1 Let d be a positive integer. With the assumptions of Proposition 1, the generating function of the form $W(z) = (\beta_0 + \beta_1 z + \dots + \beta_{N-1} z^{N-1})^{\frac{\alpha}{d}}$ approximates the fractional derivatives $D_{x\pm}^\alpha f(x)$ at x with order p and shift r if and only if the coefficients of $P(z)$ govern the linear system:

$$\sum_{j=0}^{N-1} (\lambda - j)^k \beta_j = d! \delta_{d,k}, \quad k = 0, 1, \dots, N-1 \quad (8)$$

where $\lambda = \frac{r^d}{\alpha}$, $N = p + d$ and $\delta_{d,k}$ is the Kronecker delta.

Proof: In view of Proposition 1, we have $G(z) = \frac{1}{z^\alpha} W(e^{-z}) e^{rz} = 1 + O(z^p)$. This gives

$$\begin{aligned} G(z) &= \frac{1}{z^\alpha} \left(\sum_{j=0}^M \beta_j e^{-jz} \right)^{\frac{\alpha}{d}} e^{rz} = \frac{1}{z^\alpha} \left(\sum_{j=0}^M \beta_j e^{-jz} \right)^{\frac{\alpha}{d}} e^{rz} \\ &= \frac{1}{z^\alpha} \left(\sum_{j=0}^M \beta_j e^{(\frac{rd}{\alpha}-j)z} \right)^{\frac{\alpha}{d}} = \left(\frac{1}{z^d} \sum_{j=0}^M \beta_j e^{\lambda_j z} \right)^{\frac{\alpha}{d}} \\ &= \left(\frac{1}{z^d} \sum_{j=0}^M \beta_j \sum_{k=0}^{\infty} \frac{1}{k!} \lambda_j^k z^k \right)^{\frac{\alpha}{d}} = \left(\frac{1}{z^d} \sum_{k=0}^{\infty} b_k z^k \right)^{\frac{\alpha}{d}} \\ &= \left(\frac{1}{z^d} \sum_{j=0}^M \beta_j \sum_{k=0}^{\infty} \frac{1}{k!} \lambda_j^k z^k \right)^{\frac{\alpha}{d}} = \left(\frac{1}{z^d} \sum_{k=0}^{\infty} b_k z^k \right)^{\frac{\alpha}{d}} \end{aligned}$$

$$\begin{aligned} &= \left(\frac{b_0}{z^d} + \frac{b_1}{z^d} + \dots + \frac{b_{d-1}}{z} + b_d + \sum_{k=d+1}^{\infty} b_k z^{k-d} \right)^{\frac{\alpha}{d}} \\ &= 1 + O(z^p), \text{ where } \lambda_j = \lambda - j, \quad \lambda = \frac{rd}{\alpha} \end{aligned}$$

and

$$b_k = \frac{1}{k!} \sum_{j=0}^M \lambda_j^k \beta_j, \quad k = 0, 1, 2, \dots \quad (9)$$

Since $G(z)$ is analytic, it does not have any poles and hence $b_k = 0$, for $k = 0, 1, \dots, d-1$. Moreover, since $G(0) = 1$, we have $b_d = 1$. These are the consistency condition for the GTA with generating function $W(z)$.

Now, for order $p = 1$, these conditions give the system (9) and the proof ends.

For $p > 1$, $G(z)$ reduces to

$$\left(1 + \sum_{k=d+1}^{\infty} b_k z^{k-d}\right)^{\frac{\alpha}{d}} =: (1 + X)^{\gamma} \\ = 1 + O(z^p) \quad (10)$$

where $X = \sum_{k=d+1}^{\infty} b_k z^{k-d}$ and $\gamma = \frac{\alpha}{d}$.

Expansion of $(1 + X)^{\gamma}$ in (11) gives

$$1 + \gamma X + \frac{\gamma(\gamma-1)}{2!} X^2 + \dots = 1 + O(z^p) \quad (11)$$

The term z appears in the term γX only on the left hand side of (12). This gives $b_{d+1} = 0$. The same is true for all $b_k, k = d+1, d+2, \dots, p+d-1$ by successively comparing the coefficients of $z^{k-d}, k = d+1, d+2, \dots, p+d-1$ to gain $O(z^p)$ in (12). Altogether, we have $b_k = \delta_{d,k}, k = 0, 1, 2, \dots, p+d-1$ which yield the system (9) with (10) by setting $M = N - 1 = p + d - 1$. This sets $N = p + d$.

The linear system (6) can be expressed in matrix form as

$$V = V(\lambda_0, \lambda_1, \dots, \lambda_{N-1}) \mathbf{b} = \mathbf{d}, \quad (12)$$

with $\mathbf{b} = [\beta_0, \beta_1, \dots, \beta_{N-1}]^T$, $\mathbf{d} = [0, 0, \dots, d!, 0, \dots, 0]^T$ and the Vandermonde matrix V of parameters $\lambda_0, \lambda_1, \dots, \lambda_{N-1}$ whose j^{th} column is $[1, \lambda_j, \lambda_j^2, \dots, \lambda_j^{N-1}]^T$ and the determinant is given by $|V| = \prod_{i>j} (\lambda_i - \lambda_j)$.

Theorem 2. Let $\alpha > 0, d$ be a positive integer and $f(x)$ be a sufficiently smooth function such that $D^d f(x)$ exists and $D_{x\pm}^\alpha f(x)$ is defined. For a Grünwald type approximation (2) for $D_{x\pm}^\alpha f(x)$ of order p and shift r with the generating function in the form (5), the coefficients β_j are given by

$$\beta_j = \frac{N_j}{D_j}, \quad j = 0, 1, \dots, N-1 \quad (13)$$

where $N = p + d$,

$$N_j = \sum_{\substack{0 \leq m_1 < m_2 < \dots < m_{p-1} \leq N-1 \\ m_i \neq j}} \prod_{k=0}^{p-1} (\lambda - m_k),$$

and

$$D_j = \frac{(-1)^d}{d!} \prod_{\substack{m=0 \\ m \neq j}}^{N-1} (j - m).$$

Moreover, the leading error coefficient of the approximation is given by

$$R = \frac{\alpha}{N! d} \sum_{j=0}^{N-1} (\lambda - j)^N \beta_j. \quad (14)$$

Proof: Solving the Vandermonde system (13) by Cramer's rule, we get

$$\beta_j = \frac{|V_j(\mathbf{d})|}{|V(\lambda_0, \lambda_1, \dots, \lambda_{N-1})|} \quad (15)$$

where $j = 0, 1, \dots, N-1$, $V_j(\mathbf{d})$ is the matrix obtained from $V(\lambda_0, \lambda_1, \dots, \lambda_{N-1})$ by replacing its j^{th} column with \mathbf{d} . For evaluating $|V_j(\mathbf{d})|$, observe that the j^{th} column has only one non-zero entry at the d^{th} place. Hence, pivoting with the j^{th} column, we have $|V_j(\mathbf{d})| = (-1)^{j+d} |V_{j,d}|$, where $|V_{j,d}|$ is the determinant of the matrix obtained from removing the j^{th} column and d^{th} row from the Vandermonde matrix V and is given by

$$|V_j(\mathbf{d})| = (-1)^{j+d} d! \prod_{\substack{m > n \\ m, n \neq j}} (\lambda_m - \lambda_n) N_j$$

By rearranging the Vandermonde determinant as

$$V = \prod_{\substack{m > n \\ m, n \neq j}} (\lambda_m - \lambda_n) (-1)^j \prod_{\substack{m=0 \\ m \neq j}}^{N-1} (\lambda_m - \lambda_j)$$

we have with $\lambda_m - \lambda_j = j - m$, and substituting in (16), we obtain (14).

From (12), and the leading error coefficient is given by the non-zero coiefficient of z^p which is $b_{p+d} = b_N = \frac{1}{N!} \sum_{j=0}^{N-1} (\lambda - j)^N \beta_j$.

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III. RESULTS AND DISCUSSION

Compact FD formulas are those that use the minimum number of grid points for a specified derivative order and order of approximation. It is the compact FD forms the are extensively used in applications. There are known compact FD forms in forward (right), backward (left) and central (symmetric) flavours. There are also some other compact FD forms such as shifted and staggered. The presented algorithm gives all these forms. The input parameters for compact finite difference forms for an integer order derivative is chosen as $d = \alpha$. The order of approximation can be independently chosen as desired. All the above mentioned flavored forms are obtained through the shift parameter r as given in Table I. When $d < \alpha$ with $\frac{d}{\alpha}$ integer, we obtain non-compact FDAs.

TABLE I. CHOICES OF SHIFT r FOR VARIOUS FINITE DIFFERENCE FORMULAS FOR INTEGER DERIVATIVES

$\alpha = d$	Backward	Forward	Central	Shifted	Staggered
Shift r	$r = 0$	$r = N$ -1	$r = \frac{N-1}{2}$	any integer r	any real r

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INSTITUTION WHERE RESEARCH WAS CARRIED OUT

Postgraduate Institute of Science, University of Peradeniya, Peradeniya, Sri Lanka

A STUDY ON RAINBOW COLORING (SANDAT GRAPH)

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I. INTRODUCTION

Graphs are one of the prime objects of study in discrete mathematics. In general, a graph is represented as a set of vertices (nodes or points) connected by edges (arcs or lines). Graphs are therefore mathematical structures used to model pairwise relations between objects. A simple graph G consists of a non-empty finite set $V(G)$ of elements called vertices and a finite set $E(G)$ of distinct unordered pairs of distinct elements of $E(G)$ called edges (Wilson, 2018).

Graph labeling is one of the most intriguing areas in graph theory and Graph coloring is a special case of graph labeling with assigning colors to edges or vertices of the graph. Graph coloring has initially started with the problem of coloring the countries of a map having the property that no two countries that have a common border receive the same color. The famous four-color problem was conjectured by Francis Guthrie (1831-1899) in 1852 and he observed that he could color the countries in a map of England with only four colors (Chartrand et al., 2008).

Let G be a nontrivial connected graph on which is defined a coloring $c : E(G) \rightarrow \{1, 2, \dots, k\}$, $k \in \mathbb{N}$, of the edges of G , where adjacent edges may be colored the same. A $u-v$ path P in G is a rainbow path if no two edges of P are colored the same. The graph G is rainbow-connected (with respect to c)

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if G contains a rainbow $u-v$ path for every two vertices u and v of G . In this case, the coloring c is called a rainbow coloring of G and it is a rainbow k -coloring if k colors are used. Rainbow connection number $rc(G)$ of a graph G is the minimum k for which there exists a rainbow k -coloring of the edges of G (Chartrand et al., 2008).

In our study, we have introduced the extended version of Sandat graph and discussed the rainbow connection number of symmetric and non-symmetric of the higher order extension of Sandat graph and also methods for the rainbow coloring of the above graph. In the symmetric subdivision, the number of vertices for each petal is even and in the non-symmetric subdivision it is odd.

II. METHODOLOGY

A. Definition: Sandat Graph

The Sandat graph on $3n + 1$ vertices, denoted by $St(n)$, is a graph with the vertex set $V(St(n)) = \{r, s_{ij}, t_i | 1 \leq i \leq n \text{ and } 1 \leq j \leq 2\}$ and the edge set $E(St(n)) = \{rt_i, rs_{ij}, s_{ij}t_i | 1 \leq i \leq n \text{ and } 1 \leq j \leq 2\}$ [4].

For example, we can see a Sandat graph $St(5)$ on Fig. 1.

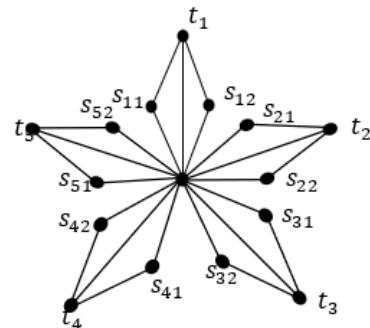


Fig. 1. Sandat Graph of $St(5)$

Using the Sandat graph we have introduced the symmetric and non-symmetric higher order extended version of Sandat graph.

III. RESULTS AND DISCUSSION

A. Definition: The Higher Order Extended Version of Sandat Graph

A higher order extension of Sandat graph $SSt_m(n)$ having n number of petals can be obtained using the symmetrical subdivisions of having $2(2 + m); m \in \{1, 2, 3, \dots\}$ vertices for each petal and with the vertex set $V(SSt_m(n))$ and edge set $E(SSt_m(n))$ denoted by,

$$V(SSt_m(n)) = \{r, s_{ij}^h, t_i; 1 \leq i \leq n, 1 \leq j \leq 2, 1 \leq h \leq m+1\}$$

$$E(SSt_m(n)) = \{rt_i, rs_{ij}^h, t_i s_{ij}^1, s_{ij}^k s_{ij}^{k+1}; 1 \leq i \leq n, 1 \leq j \leq 2, 1 \leq h \leq m+1, 1 \leq k \leq m\}$$

For example, we can illustrate the $SSt_2(n)$ graph as in Fig. 2.

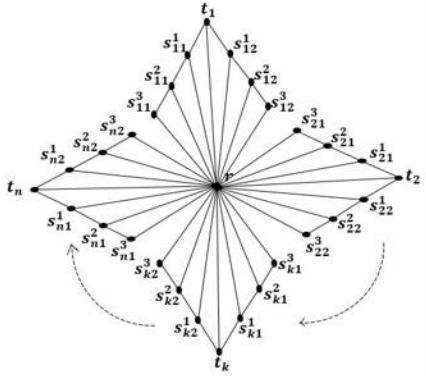


Fig. 2. The Graph of $SSt_2(n)$

B. Definition: The non-symmetric of the higher order extension of Sandat graph $SSt'_2(n)$.

The non-symmetric of the higher order extension of Sandat graph $SSt'_m(n)$ can be obtained having $2(2 + m) - 1; m \in \{1, 2, 3, \dots\}$ vertices for each petal and with the vertex set $V(SSt'_m(n))$ and edge set $E(SSt'_m(n))$ denoted by,

$$V(SSt'_m(n)) = \begin{cases} r, s_{ij}^h, t_i & 1 \leq i \leq n, 1 \leq h \leq m+1 \\ s_{ij}^h & 1 \leq i \leq n, 1 \leq h \leq m \end{cases}$$

$$E(SSt'_m(n)) = \begin{cases} rt_i, rs_{ij}^h, s_{ij}^k s_{ij}^{k+1} & 1 \leq i \leq n, 1 \leq h \leq m+1, 1 \leq k \leq m \\ rs_{ij}^h, s_{ij}^k s_{ij}^{k+1} & 1 \leq i \leq n, 1 \leq h \leq m+1, 1 \leq k \leq m-1 \end{cases}$$

For example, we can illustrate the non-symmetric of the higher order extension of Sandat graph $SSt'_2(n)$.

Algorithm to color the graph $SSt_m(n)$, where $n > 1$

Let's take three colors as c_1, c_2 and c_3 . Note that $i, j, h, k \in \{1, 2, 3, \dots\}$. The following two methods give the rainbow coloring of $SSt_m(n)$.

Algorithm 01:

Step 1: Select one petal and start to color with an end edge of rs_{ij}^h (choose the maximum h). (Say c_1)

Step 2: Starting from the top edge move in clockwise direction (or anticlockwise) and color all the edges rs_{ij}^h and $t_i r$ edges in the petal that you choose in step 1 with the order of the colors c_1, c_2 and c_3 .

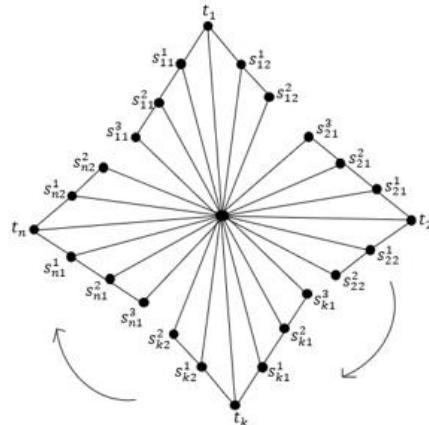


Fig. 3. The Graph of $SSt'_2(n)$

Step 3: Now it will remain only the edges $t_i s_{ij}^h$ and $s_{ij}^k s_{ij}^{k+1}$ to color. Now select the smallest triangle having only three edges and color the remaining edge (choose the color among c_1, c_2, c_3) having the property that adjacent edges not having the same color.

Step 4: Use the same steps from 1-3 for other petals too.

Algorithm 02:

Choose any edge among rs_{ij}^h edges as the initial and color all the edges rs_{ij}^h in the graph with the order of colors c_1, c_2 and c_3 in clockwise or anticlockwise direction. Now select the smallest triangle having only three edges and color the remaining edge (choose the color among c_1, c_2, c_3) having the property that adjacent edges not having the same color.

Algorithm 03:

Step 1: Take three colors as c_1, c_2 and c_3 .

Step 2: Choose any edge rt_i or rs_{ij}^k in any petal and color with c_1 .

Step 3: Starting from the colored edge move in clockwise direction (or anticlockwise) and color all the other edges rt_i and rs_{ij}^k in the order of c_1, c_2 and c_3 colors.

Step 4: Now it will remain only the edges $t_i s_{ij}^h$ and $s_{ij}^k s_{ij}^{k+1}$ to color. Now select the smallest triangle having only three edges and color the remaining edge (choose the color among c_1, c_2 and c_3) having the property that adjacent edges not having the same color.

Theorem 1

For any integer $n \geq 2$, let $SSt_m(n)$ be a higher order extension of Sandat graph on $2(2 + m)$ vertices. Then $rc(SSt_m(n)) = 3$.

Proof:

Let x and y be two different vertices of $SSt_m(n)$. We can prove that there exists an $x - y$ rainbow path for any $x, y \in V(SSt_m(n))$. If x and y are adjacent, then it is clear that there is a $x - y$ rainbow path. If x and y are not adjacent, then for

some i and j in $\{1, 2, 3, \dots, n\}$ and p and q in $\{1, 2, 3, \dots, n\}$, we obtain a rainbow path.

Since there is an $x - y$ rainbow path for every $x, y \in V(SSt_m(n))$, we have,

$$rc(SSt_m(n)) \leq 3$$

Suppose $rc(SSt_m(n)) = 2$. In this case the length of the path between any two vertices must be two, to have the rainbow coloring between any two vertices. But we can show that the rainbow connection number is not equal to two. Hence,

$$rc(SSt_m(n)) = 3.$$

For the complete proof (Dheerasinghe et al., 2024).

Corollary 1: Rainbow connection number of $SSt_1(1)$ is two.

Proof:

As per given in the figure 4 it is clear that we can color the graph with 2 colors having at least one rainbow path for every 2 vertices.

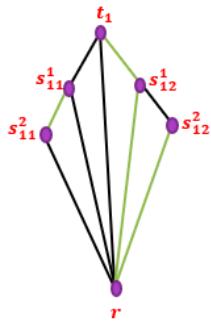


Fig. 4. Rainbow coloring of $SSt_1(1)$

For the complete proof (Dheerasinghe et al., 2024).

Corollary 2: Rainbow connection number of $SSt_m(1)$ is three, where $m > 1$.

Proof:

Consider the two colors and the vertices (t_1, s_{11}^k) and (t_1, s_{12}^k) , where $k = \{2, 3, \dots\}$. Table 1 shows the edge coloring of the vertices t_1, s_{11}^k and s_{12}^k .

TABLE I: RAINBOW PATH AND EDGE COLORING

x	y	Minimum Path	Edge Coloring
t_1	s_{11}^k	(t_1, r, s_{11}^k)	$(t_1, r) - \text{color 1}$ $(r, s_{11}^k) - \text{color 2}$
t_1	s_{12}^k	(t_1, r, s_{12}^k)	$(t_1, r) - \text{color 1}$ (already colored) $(r, s_{12}^k) - \text{color 2}$
s_{11}^k	s_{12}^k	(s_{11}^k, r, s_{12}^k)	$(s_{11}^k, r) - \text{color 2}$ (already colored) $(r, s_{12}^k) - \text{color 2}$ (already colored)

The table 1 shows that the rainbow connection number cannot be 2 and hence,

$$rc(SSt_m(1)) \geq 3 \text{ when } m > 1.$$

Algorithm 1 can apply for this petal and hence can use three colors to have the rainbow coloring. Also, this graph can color from 3 colors to p number of colors where p is the total number of edges.

$$\text{Hence } rc(SSt_m(1)) = 3 \text{ when } m > 1.$$

For the complete proof (Dheerasinghe et al., 2024).

Theorem 2

For any integer n at least two, let $SSt'_m(n)$ be a non-symmetric higher order extension of Sandat graph on 2 ($2 + m$) – 1 vertices. Then $rc(SSt'_m(n)) = 3$ when $n \geq 2$.

Proof:

The proof of theorem 1 is holds for this.

Corollary 3: Rainbow connection number of $SSt'_m(1)$ can find from the following.

$$rc(SSt'_m(1)) = \begin{cases} 2 & \text{when } m \in \{1, 2\} \\ 3 & m \geq 3 \end{cases}$$

Proof:

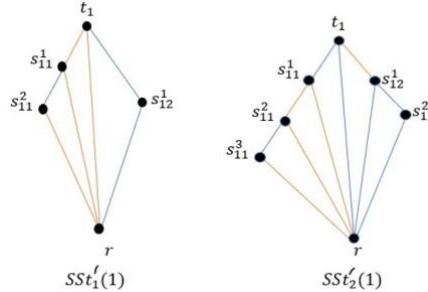


Fig. 5. Rainbow coloring of $SSt'_1(1)$ and $SSt'_2(1)$

The above figure shows that the rainbow connection number of the graphs $SSt'_1(1)$ and $SSt'_2(1)$ are two. For the graph $SSt'_m(1)$ when $m \geq 3$ the rainbow connection number is three and we can prove this using the same proof as given for the theorem 1.

IV. CONCLUSION

Graph coloring is a versatile mathematical concept that transcends its theoretical roots to have a profound impact on various practical domains. From map coloring to computer science and beyond, its applications are diverse and critical to solving real-world problems efficiently. In the present study, the rainbow connection number of symmetric and non-symmetric of the higher order extension of Sandat graph was established. In both symmetric and non-symmetric higher order extension of Sandat graph is three only when the number of petals greater than or equal to 2. Moreover, the rainbow connection number of the above graph has discussed when the number of petals equal to 1. Three algorithms have

introduced which can use to have the rainbow coloring of the above graphs.

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PHYSICS

GROWTH AND CHARACTERISATION OF THERMALLY EVAPORATED CdS THIN FILMS FOR CdS/CdTe THIN FILM SOLAR CELLS

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I. INTRODUCTION

Cadmium Sulfide/Cadmium Telluride (CdS/CdTe) solar cells represent a promising photovoltaic technology that converts sunlight into electrical energy. These solar cells utilise a thin film of CdTe as the absorber layer, which efficiently captures solar energy due to its near-ideal bandgap of approximately 1.45 eV, well-suited to the solar spectrum. The CdTe layer, typically deposited via techniques like thermal evaporation or close-space sublimation, absorbs photons, generates electron-hole pairs, and initiates the photovoltaic process.

The CdS layer in CdS/CdTe solar cells plays a crucial role in the overall performance of the device. The CdS layer serves as an optical window layer. It is transparent to the visible spectrum of sunlight and allows photons to pass through and be absorbed by the underlying CdTe layer. It forms a heterojunction with the CdTe layer. This p-n junction is essential for separating the photogenerated electron-hole pairs, which is a critical step in converting light into electricity.

The significance of CdS/CdTe solar cells lies in their high efficiency, cost-effectiveness, and scalability, making them a core technology in the global pursuit of sustainable energy solutions. With their ability to harness solar energy with remarkable efficiency and low production cost, CdS/CdTe

solar panels play a crucial role in advancing the transition to clean, renewable energy sources.

The aim of the current study was to fabricate CdS/CdTe solar cells with high efficiencies by optimising the deposition conditions of thermally evaporated CdS layer (TE-CdS) and introducing novel concepts for CdS layer deposition.

The study was conducted with the following specific objectives:

- i. Optimisation of CdS Thermal Evaporation Parameters.
- ii. Seed-Assisted Growth of Thermally Evaporated CdS Layer
- iii. Optimisation of CdS Graded Annealing Parameters

II. METHODOLOGY

A. TE-CdS Thin Films for CdS/CdTe Solar Cells: Effect of Substrate Temperature on CdS Layer.

Thermal evaporation of CdS was conducted on cleaned fluorine-doped tin oxide (FTO) substrates by varying substrate temperatures (50–250 °C) using 4N CdS powder in a high vacuum condition. Deposited films were annealed and used for characterisations and solar cell fabrication. Morphological analysis was performed using scanning electron microscope (SEM), structural analysis was performed using Grazing Incidence X-ray Diffraction (GIXRD), and electrical properties were studied via current-voltage (*I-V*), capacitance-voltage (*C-V*), and electrochemical impedance (EIS) measurements. Optical behaviour was characterised using UV-Vis spectrophotometers. CdTe layers were deposited on CdS to form solar cells, followed by CdCl₂ deposition, annealing, and etching. Back contacts (Cu/Au stacks) were deposited, and solar cells were annealed before electrical characterisation. *I-V* and external quantum efficiency (EQE) measurements were conducted, and solar cell simulations were performed using SCAPS-1D software for comparison with experimental results.

B. Effect of CdS Layer Thickness on Thermally Evaporated CdS/CdTe Solar Cell Efficiency.

CdS thin films were deposited on FTO using the thermal evaporation technique in a high vacuum by varying deposition durations (1 to 30 minutes) to achieve different thicknesses. Deposited thin films were post-heat treated and used for characterisation and solar cell fabrication. Surface topology was analysed using atomic force microscopy

(AFM), and thickness measurements were obtained from AFM line profiles. GIXRD diffractograms were obtained using a Bruker D8 Advance XRD system. The electrical properties of CdS layers were studied using *I-V* and *C-V*. Optical characteristics were analysed using a UV-Vis spectrophotometer. CdS/CdTe solar cells were fabricated, as mentioned previously. Electrical characterisations were performed using a solar simulator, and the EQE spectrum was obtained using a PVE300 photovoltaic QE system.

C. Medium-Vacuum Thermal Evaporation of CdS:O Thin Films Towards Industrial CdS/CdTe Solar Cells

This study employed medium-vacuum thermal evaporation to deposit oxygenated CdS (CdS:O) thin films on FTO substrates by optimising substrate temperature, source temperature, and oxygen concentration in the ambient environment. Substrate temperature optimisation for CdS deposition was conducted by varying temperatures from 100 to 300 °C, maintaining a chamber pressure of 2×10^{-4} Torr with 16% Oxygen at Argon ambient. Oxygen concentration optimisation involved varying concentrations from 10% to 30%, with Argon as the carrier gas. Source temperature optimisation was performed by varying temperatures from 475 to 575 °C at a fixed substrate temperature of 150 °C. The CdS/CdTe solar cells were fabricated by depositing a 5 μm thick CdTe layer on the CdS:O layer using close-spaced sublimation, followed by previously described post-treatments. CdS:O layer characterisations included surface morphology analysis using SEM, structural analysis using GIXRD, optical behaviour analysis using UV-Vis spectrophotometer, and electrical characterisations using a solar simulator.

D. Seed Assisted Thermal Evaporation of CdS:O: Optimisation of Electrodeposited Seed Layer

The methodology was initiated with preliminary studies on the electrodeposition of thin CdS layers on FTO with a three-electrode system to determine suitable potential ranges for electrodeposition. Constant-potential and pulse-potential deposition methods were employed, and successfully deposited films were annealed and characterised. Applied potential and duration optimisations of electrodeposited CdS (ED-CdS) seed layers were conducted using a three-electrode system with varying parameters. The deposited thin films were washed, dried, annealed, and subjected to characterisations. Characterisations included surface morphology analysis using SEM, structural analysis using GIXRD, surface chemistry analysis using X-ray photoelectron spectroscopy (XPS), and optical behaviour analysis using UV-Vis spectroscopy. CdS/CdTe solar cells were fabricated by depositing TE-CdS layers on the ED-CdS seed layers, followed by CdTe layer deposition and post-deposition treatments. Solar cells were electrically characterised using a solar simulator. The optimum applied potential and deposition duration were determined based on characteristic observations for subsequent studies.

E. Graded Annealing of Thermally Evaporated CdS:O Thin Films for CdS/CdTe Solar Cells

The methodology entails the graded annealing of thermally evaporated CdS:O thin films for CdS/CdTe solar cells. Initially, FTO substrates were cleaned and prepared for thermal evaporation of CdS:O thin films. The deposition process was conducted with pre-optimised conditions. Subsequently, the CdS:O films underwent annealing at 450 °C with predefined temperature profiles. CdS/CdTe solar cells were then fabricated as described previously. Characterisation of CdS included SEM for morphology, UV-Vis spectroscopy for optical behaviour, and electrical measurements using a solar simulator for assessing the fabricated solar cells' properties.

III. RESULTS AND DISCUSSION

The optical characterisation of CdS thin films revealed that increasing the substrate temperature up to 175 °C resulted in higher transmittance values beyond 500 nm, indicating improved crystallinity. Additionally, the blue shift increased with the substrate temperature up to 175 °C, allowing transmission of additional spectral wavelengths. Bandgap values increased up to 175 °C before decreasing. Morphologically, films deposited at 175 °C displayed optimal properties with lower cluster size and roughness. Structural analysis confirmed hexagonal CdS formation, with a dominant orientation of (002) plane. Electrical characterisation showed increased J_{SC} and V_{OC} values up to 175 °C for CdS photoelectrochemical (PEC) cells, attributed to improved crystallinity. CdS/CdTe solar cell performance mirrored CdS properties, achieving the highest efficiency of 3.76% at 175 °C. Simulation results validated experimental findings, suggesting 175 °C as the optimal substrate temperature for CdS thin film growth at high vacuum.

From the results of the second study, the optical characterisation revealed that increasing the thickness of the deposited CdS thin films led to a red-shift in the absorption edges, with films over 210 nm exhibiting relatively constant absorption properties. The highest optical bandgap was observed for the 210 nm thick CdS film, indicating optimal window layer properties. Topologically, the optimum properties are obtained by 210 nm thickness. Electrical characterisation using PEC cells demonstrated that thinner films had higher carrier concentrations and current densities. The CdS film thickness of 210 nm demonstrated optimal electrical properties. Structural characterisation via GIXRD confirmed that film thickness up to 210 nm exhibited enhanced crystallinity before decreasing, with the hexagonal phase dominating. Fabricated CdS/CdTe solar cells using the 210 nm thick CdS layer demonstrated the highest efficiency of 4.43%, with simulated efficiencies supporting the experimental findings.

The optical characterisation of the third study revealed that substrate and source temperatures influence absorption edges. These shifts can be attributed to variations in bandgap and Urbach energy. Morphological analysis indicated that substrate temperature affects the particle size and morphology, with nanowire-like structures observed at

higher temperatures. Structural analysis via XRD revealed hexagonal polycrystalline CdS films with crystallographic parameters influenced by temperature variations. The electrical properties of CdS/CdTe solar cells were studied, showing efficiency enhancements at specific deposition conditions. Notably, the highest efficiency of 9.31% was achieved at a substrate temperature of 150 °C, oxygen concentration of 20%, and source temperature of 500 °C. The study concludes that optimising these parameters enhances the performance of CdS/CdTe solar cells.

The fourth study focused on optimising electrodeposition parameters for CdS seed layers to enhance the fabrication of CdS/CdTe solar cells. Preliminary cyclic voltammetry analysis guided the selection of the electrodeposition potential range. Optical characterisations revealed that seed layers deposited at -680 mV exhibit optimal properties, including a significant blue shift and higher bandgap. Morphological analyses showed the largest seed size at -680 mV, enhancing the CdS/CdTe junction area. Structural analysis and XPS results support the findings of optical properties. These optimised seed layers significantly improved the electrical properties of ED-CdS/TE-CdS:O/CdTe solar cells, with a maximum efficiency of 10.4%. The study underscores the importance of precise control over electrodeposition parameters for enhancing CdS/CdTe solar cell performance.

The fifth study investigated the annealing temperature profiles for CdS:O thin films and revealed crucial insights into optimising CdS/CdTe solar cell efficiency. An initial optimisation study determined 450 °C as the optimal annealing temperature. Subsequent annealing temperature profile optimisations focused on the cooling-down, heating-up, and constant temperature regions. For the cooling-down phase, a 25 °C/min gradient was found optimal, leading to a blue shift in absorption edge and a bandgap increment, resulting in an efficiency of 8.38%. Likewise, a 60 °C/min heating-up rate exhibited a higher bandgap, correlating with an efficiency increase of 9.95%. Furthermore, an optimal constant temperature duration of 1200 s achieved the highest bandgap and a maximum average efficiency of 10.73% in CdS/CdTe solar cells. These findings highlight the significant impact of annealing parameters on CdS:O thin film properties and the subsequent performance of CdS/CdTe solar cells, emphasising the importance of precise annealing temperature control for enhanced device efficiency.

IV. CONCLUSIONS

This research explores the deposition and optimisation of hexagonal n-CdS thin films for integration into CdS/CdTe thin-film solar cells. This research explains key factors influencing film properties and device performance through systematic investigations across multiple studies. The first study highlights the importance of substrate temperature in the thermal evaporation of CdS thin films, with films deposited at 175 °C exhibiting better characteristics of the CdS layer and resulting in solar cells with efficiencies up to 3.76%. The second study underscores the importance of optimising CdS layer thickness, achieving efficiencies of 4.43%. The third study introduces oxygenated CdS layers, enhancing device performance to an average efficiency of 9.31%. The fourth study focuses on electrodeposition parameters of the ED-CdS seed layer, leading to improved TE-CdS:O layer properties and solar cell efficiencies of up to 10.4%. Finally, the fifth study investigates the influence of the annealing parameters, culminating in a maximum average efficiency of 10.73%, highlighting the significant impact of precise annealing on device efficiency.

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SYNTHESIS AND CHARACTERIZATION OF Sb₂S₃ AND FABRICATION OF EFFICIENT THIN FILM SOLAR CELLS

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I. INTRODUCTION

The large-scale production of solar cells worldwide consists of single crystal, multi-crystalline, and thin-film amorphous Si solar cells. The cost of single-crystal Si solar cells is determined by the thickness (250-300 μm) of the Si wafer. Therefore, this problem can be solved through the utilization of low-cost materials and technologies. In the last decades, the study of thin films has increased intensively because of the abundance of their raw materials. Therefore, they can be obtained easily at a lower cost. Generally, a number of thin films are used as absorbers in solar cells, such as CdS, CdTe, CIGS, CZTS, Sn(S,Se), CuO, CuSbS, and Sb₂(Se,S)₃.

Among the binary compound semiconductors, Sb₂S₃ has reached a very strong place in solar cell applications due to its admirable optical and electrical properties. Since the bandgap of Sb₂S₃ perfectly matches the required bandgap values for the top sub cell in Si-based tandem dual-junction solar cells, it gives a maximum theoretical PCE of ~ 42% (Ghosh. et al., 1979; De Vos. et al., 1980).

Much effort has been made by the researchers to improve the device performance by controlling the size, morphology, and crystallinity of the films. Because of the various deposition methods, nature of precursors, growth conditions, and parameters, the devices give different values of power conversion efficiency (Englman. et al., 2015; Noh et al., 2018).

To enhance the performance of solar devices, the compactness of each layer is essential. Therefore, choosing the material for each layer of a completed solar cell seems like a challenge.

II. METHODOLOGY

A. Optimization of electron transport layer (ETL).

A mixture of di-ethanolamine, butan-1-ol, TTIP used to prepare different concentrations of TiO₂ precursor solution. These solutions were deposited on the cleaned FTO by spin coating technique. Then, SbCl₃ and TU were added to 2-methoxyethanol to prepare the Sb₂S₃ precursor solution, and was spin-coated on a various spinning cycles of compact TiO₂. After spinning, the cells were heated for 1 minute at 160 °C on the hot plate. After cooling, Sb₂S₃ coated FTO substrates were annealed inside the tube furnace at 280 °C under N₂ for 10 minutes, followed by natural cooling conditions. Following this, P3HT solution was spin coated on the Sb₂S₃ layer. A 70 nm thick Ag layer was deposited on top of the P3HT layer by thermal evaporation technique at 1×10⁻⁵ mBar pressure. The active area on the cell was limited to 0.18 cm² by using a mask.

B. Improving the performance of the devices based on various factors on Sb₂S₃ films.

The optimized layer of ETL was used for further examination of Sb₂S₃. Based on temperature: 1.5 mmol of thiourea (TU) and 1 mmol of SbCl₃ were mixed with 1 ml of 2-methoxyethanol for Sb₂S₃ precursor. Spin coated cells were heated at different temperature of 155, 170, 185, and 200 °C.

Based on concentration: 1.5 mmol of TU was mixed with different volumes of 2-methoxyethanol such as

0.5, 1, and 1.5 ml and stirred for 5 mins. Then, 1 mmol of SbCl₃ was added to TU solution and stirred for 30 mins.

Based on spinning cycle: 1.5 mmol of TU was mixed with 1 ml of 2-methoxyethanol and stirred for 5 mins. Then, 1 mmol of SbCl₃ was added to TU solution and stirred for 30 mins. The prepared solution spin coated at various spinning cycles.

Based on spinning speed: 1.5 mmol of thiourea (TU) was mixed with 1 ml of 2-methoxyethanol and stirred for 5 mins. Then, 1 mmol of SbCl₃ was added to TU solution and stirred for 30 mins. The prepared solution spin coated at various spinning speeds.

C. Examine the influence of Hole Transport Materials (HTM) in Sb₂S₃ solar cells by using different HTMs

A clear solution of 0.25 mM TiO₂ precursor was prepared using 50 ml of di-ethanolamine and 910 ml of butan-1-ol 75 ml of TTIP. The prepared 1 M Sb₂S₃ solution spin coated at 5000 rpm for 30 s. After spinning, the cells were heated for 1 minute at 150 °C on the hot plate. After cooling, coated cells were annealed inside the tube furnace at 280 °C under N₂ for 10 minutes, followed by natural cooling conditions. A 20 mg of CuSCN was dissolved in 1 ml of 2-Ethylsulfide to prepare the CuSCN solution. And, the Spiro solution was prepared by using 3.6 mg of Spiro-OMeTAD in 100 ml of chlorobenzene, 1.0 ml of Li⁺ solution, and 1.5 ml of TBP. The Li⁺ solution was prepared by adding 520 mg of bis(trifluoromethane sulfonyl) imide lithium salt to 1 ml of acetonitrile. The prepared solutions of the above HTMs were spin-coated on Sb₂S₃ at 3000 rpm for 30 s. the coated cells were heated on the hot plate at 100 °C for 20 minutes. In this study, some sets of cells were fabricated with CuSCN, Spiro, CuSCN/Spiro, and Spiro/CuSCN as HTL separately.

III. RESULTS AND DISCUSSION

The TiO₂ precursors with different concentrations were directly deposited on FTO glass by a spin coating method and used as sample cells while FTO was used as a reference cell for the UV-Vis spectroscopy. It shows the optical absorption spectra for TiO₂ layers with various spinning cycles (1-5) for different concentrations of 0.34 and 0.25 mM, respectively. In both concentrations, the absorption plots exhibit an increasing peak with the increasing number of spinning cycles. Each TiO₂ layer exhibits a more absorption of light in the UV region (300-350 nm). Because the wide bandgap materials absorb only the UV light. Also, the peaks appear at about 310 nm and there is no significant difference at the peak position. That implies that the pure compound is fabricated along the whole process, which means the appropriate condition is applied for the fabrication of TiO₂.

In 0.34 mM precursor, the solar cell parameters of the devices, the solar device with three cycles of TiO₂ layer exhibited the highest performance among others as J_{sc} of 14.93 mA cm⁻², Voc of 575.8 mV, FF of 36.7%, which yielded a PCE of 3.16%. The solar cell efficiency was improved by increasing the number of spinning cycles of TiO₂ up to three cycles then followed by a performance drop.

In 0.25 mM precursor, the solar cell parameters of the devices with four cycles of TiO₂ layer exhibited the highest performance among others as J_{sc} of 13.82 mA cm⁻², Voc of 592.0 mV, FF of 43.0%, which yielded a PCE of 3.52%. The solar cell efficiency was improved by increasing the number of spinning cycles of TiO₂ up to four cycles and again was decreasing above four cycles. The TiO₂ film with four cycles in 0.25 mM shows good performance compared to three cycles of TiO₂ in 0.34 mM, whereas, the power conversion efficiency was improved by 11%. The low concentration solution of TiO₂ (0.25 mM) gives a compact layer to lead the device performance. It was confirmed by an improved fill factor of ~ 17%.

The crystal structure of Sb₂S₃ was confirmed by X-ray diffraction (XRD), and diffraction peaks at 15.65°, 17.54°, 25.04°, 29.26°, 32.37°, 33.42°, 46.84°, 54.45°, 64.71°, and 71.24° can be indexed to orthorhombic stibnite structure (Entry #01-073-0393) crystal planes of (200), (201), (103), (211), (212), (013), (015), (603), (712), and (803).

The device with Sb₂S₃ at 185/280 °C exhibits higher efficiency (3.04%) than others under the N₂ environment. It was ~ 5 times greater than the direct annealing at 280 °C. The O is found to prefer occupying one of the two S sites and forming Os defect. However, the dominant donor Vs is filled due to the low formation energy of Os and thus the Fermi level should be pushed down significantly because the concentration of electrons carriers should decrease apparently as the dominant donor disappears. Therefore, oxidized Sb₂S₃ can increase the p-type conductivity of Sb₂S₃, which improves its performance. The concentration, spinning cycles, and spinning speed significantly affected the performance of the solar cells.

The HOMO level of P3HT and spiro are sufficiently higher than that of Sb₂S₃, ensuring the efficient extraction of hole charge carriers at HTL/Sb₂S₃. It was found that spiro possesses an obvious better performance than P3HT with increasing J_{sc} from 14.05 to 15.6 mA cm⁻², leading to 2.5% of higher efficiency.

IV. CONCLUSIONS

In this study, the major components of solid-state Sb₂S₃ solar cells were investigated based on various parameters such as ETL, light harvester, and HTL. The presented results of solar cell based on TiO₂ shows that the thickness and compactness of ETL affect the solar device performance. The devices exhibit the best efficiencies of 3.16% and 3.52% for three cycles of 0.34 mM and four cycles of 0.25 mM of TiO₂ precursors, respectively. It was noted that the precursor concentration and the number of spinning cycles have an effect on the thickness of ETL as well as compactness, which contributed to the good performance. Since the annealing process has a major influence on the surface morphology and crystallinity of the films it affects the device performance. The Sb₂S₃ device in the two-step annealing process improved the efficiency by 2.8 times higher than the direct annealing of Sb₂S₃. Thereafter, the film quality of the two-step annealing process was studied at the appropriate temperature. The pre-

heating process inside the N₂ environment produced pure Sb₂S₃ films which increased the current. However, the cell in the air increased the open-circuit voltage to enhance the overall performance. Here, a less Sb₂O₃ film on the surface is positively working in our system. The bandgap energy of the films was in the range of 1.64-1.74 eV. Since the theoretical efficiency limit of a single p-n junction solar cell is 28.64% with ~ 1.7 eV bandgap, our films were attractive to fabricate the solar devices. The overall cell performance was improved with HTLs. Both P3HT and Spiro were highly effective in our system. However, the improved interface directly lead to the efficiency, which was ensured by the surface images of Spiro and P3HT. In this system, the most efficient hole transfer process occurs with spiro samples. Also, it was noted that the performance of the Spiro-OMeTAD could be dependent due to the additives. We concluded that the extraction of charge transfer depends on the band alignment and ability of the material as well. With the final configuration of FTO/compact-TiO₂/Sb₂S₃/Spiro-OMeTAD/Ag, the power conversion efficiency reached 4.11%.

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NOVEL TiO₂/GRAPHENE AND Nb₂O₅/GRAPHENE COMPOSITE ANODES FOR LITHIUM-ION BATTERIES

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I. INTRODUCTION

Lithium-ion Batteries (LIBs) are the dominant electrochemical storage devices due to their higher coulombic efficiency, specific energy, energy density, specific power, and operating voltage. However, one major drawback of LIBs is their lower power capability, which can be addressed by using nanomaterials for electrodes, particularly anodes. Many research groups have successfully used modified nanomaterials to enhance the rate capability of LIB anodes.

This research has been implemented to make nano transition metal oxides (TMOs), specifically TiO₂ and Nb₂O₅, due to their reasonable capacity and high lithiation potential, which potentially avoids the formation of a solid electrolyte interface layer (> 1V Vs. Li/Li⁺) that leads to the formation of lithium dendrites on the anode surface, yet the full potential of these materials has not yet been explored, especially in combination with graphene to develop metal oxide/graphene-based anode materials for LIB applications.

In Sri Lanka, vein graphite can be found with more than 95% carbon purity, making it an excellent source for synthesizing graphene. However, impurities such as iron (Fe), magnesium (Mg), and silicon (Si) must be removed as thoroughly as possible to avoid affecting the electrochemical processes in batteries. Therefore, some research groups have focused on the purity enhancement of Sri Lankan vein

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graphite, treating it with 5-20% HCl solutions at 60-65 °C, depending on the initial purity of different structural varieties.

Graphene and graphene-based nanomaterials, known as the world's most studied materials, can also be used as anode materials in LIB applications. Several groups in Sri Lanka have successfully synthesized graphene using modified Hummer's method. However, some of these methods involve hazardous materials that may cause environmental issues during large-scale synthesis. These synthesis methods can be adapted for various commercial applications of graphene. Beyond preliminary investigations, detailed studies are needed to develop graphene/metal composites using Sri Lankan natural graphite (SLNG) as a starting material. Therefore, synthesizing a low-cost metal composite/graphene material for LIB applications is essential.

Objectives of this research project:

- i. Investigating the impact of varying hydrolysis temperature, pH, oxidation time, and oxidizing agents on the functional groups in graphene oxide (GO), and optimizing GO synthesis parameters.
- ii. Studying the effect of annealing temperature on the electrical conductivity of synthesized reduced GO (rGO).
- iii. Preparing anode materials using rGO, TiO₂, and Nb₂O₅.
- iv. Fabricating anode materials for LIBs utilizing different morphologies of TiO₂ (e.g., p21, nanobelts) and Nb₂O₅ (e.g., raw Nb₂O₅, microcolumns)
- v. Preparing TMO/rGO composites and testing their performance as anodes in LIBs.

II. METHODOLOGY

A. Preparation of GO using modified Hummer's method

GO was synthesized from Sri Lankan natural vein graphite using the modified Hummer's method. The process involved oxidizing graphite powder with KMnO₄ in concentrated H₂SO₄, incorporating rigorous stirring and sonication steps to promote thorough oxidation. Following oxidation (by KMnO₄, and H₂O), the mixture was diluted, neutralized, and dried, yielding purified GO.

B. The effect of reaction temperature on the formation of functional groups of GO

The graphite powder was oxidized, and the reaction mix was subsequently divided and exposed to various temperatures (10, 30, 50, 65 and 85 °C). These conditions aimed to understand their influence on the chemical structure of GO, assessed through FTIR-ATR spectroscopy.

C. Influence of pH on the formation of functional groups in GO

The impact of pH on GO's functional groups was investigated by adjusting the pH (0, 1, 2, 3, 4, 5, 6, 7 and 8) of the oxidized graphite suspension before final purification. This method sought to determine how pH variations during processing influence the chemical properties of GO, crucial for tailoring its use in applications.

D. Characterization of the DSC series

Fabricated solar cells were irradiated with light of 1000 W m⁻² (AM 1.5) by a PEC-LO1 solar simulator, and the current-voltage (*I*-*V*) characteristics of the cells were obtained. The active area of a cell was 19 mm². Current-voltage data were measured for 3 h (at 10 min steps) under continuous irradiation. Nyquist plots of the fabricated DSCs were obtained using a Metrohm Autolab potentiostat by keeping the direct current (DC) potential set at maximum open circuit voltage (*V*_{oc}) and setting the alternative current (AC) amplitude as 0.01 V in a frequency range from 0.01 to 106 Hz.

III.RESULTS AND DISCUSSION

The major findings in this research project are as follows.

The oxidation of graphite with different oxidizing agents (KMnO₄, HNO₃) was studied. First, the formation of functional groups on the surface of graphene sheet was studied by using KMnO₄. There, the hydrolysis temperature was varied (30, 50, 65, and 85 °C) to study its effect on functional groups. FTIR analysis noted that when reaction temperature increased, the formation of carbonyl group (C=O) also increased. Next, GO solubility was studied at different pH values, and it was identified that GO can exhibit good solubility around pH 4-6. It tends to aggregate and precipitate as the pH approaches higher than 7, leading to reduced solubility.

As the next oxidizing agent, HNO₃:H₂SO₄ with 1:3 was used to study the oxidizing capability on graphite. Compared to powder XRD (PXRD) of raw graphite powder, the intensity of (002) is reduced in to some extent after treatment with H₂SO₄/HNO₃ (60 °C), confirming that the periodicity of graphene sheets is reduced. When the reaction temperature increased further (95 °C), the intensity of (002) peak was reduced, confirming that graphene layers are further peeled off from the graphite sheet due to intercalation of sulphate ions between graphene layers during sonication. FTIR-ATR analysis confirms that, the attachments of functional groups to graphitic plane are negligible compared to graphite oxidation with KMnO₄.

L-ascorbic acid (L-AA) was used to reduce GO to reduced graphene oxide (rGO) due to nontoxic and great reduction ability in contrast to hydrazine. During the reduction step of

GO into rGO, the pH of the GO solution is maintained at pH=10 because L-AA is preferred to give its best functionalities in an alkaline medium. Structural analysis of reduced graphene oxide was studied by varying the annealing temperature (100, 150, 200, 250, 300, 350, 400, 450 and 500 °C). PXRD analysis concluded that the number of graphene layers can be altered by varying the annealing temperature. Calculations show that rGO annealed 100 °C shows the lowest number of graphene layers per domain (5 layers). When the annealing temperature increased to 450 °C, graphene sheets were stacked together and shifted close to the characteristic graphite peak position in PXRD (2θ of 26.5°). The highest number of graphene layers per domain was observed for the sample annealed at 500 °C, which is 24 layers. When the temperature was further increased, the number of layers was reduced due to the oxidation of carbon to CO₂ and CO. To understand the structural defects in rGO sheets, Raman analysis was used. The largest crystallite size and lowest ID/IG was obtained for the samples annealed at 300 °C, which were 35.22 nm and 1.25. The highest conductivity was obtained for the sample annealed at 300 °C, and it was 25.39 S cm⁻¹.

Even though rGO has higher conductivity than GO, rGO cannot be combined with other materials due to poor interaction. Hence GO is getting much attention due to its unique chemical structure. Hence, the coagulation between GO and TiO₂ phenomena can be used to make GO and TiO₂ composite for LIB anode. The coagulation between commercially available TiO₂-p21 nanoparticles and optimized GO was studied by using different ratios (GO:TiO₂=1:1, 1:2, 1:3, 1:4.0, 1:4.1, 1:4.2: 1:4.3: 1:4.4: 1:4.5, 1:5, 1:6, and 1:12). For the anode fabrication TiO₂/rGO was synthesized from GO: TiO₂=1:4.0 (20:80) based on precipitation study. The fabricated TiO₂/rGO electrode (TiO₂/rGO-1) retained 85.6% of capacity after 100th cycle. Moreover, the composite material delivered 99.6 mA h g⁻¹ at a current rate as high as 5000 mA g⁻¹. For comparison, another electrode was fabricated using just mixing TiO₂, rGO, CB and CMC mechanically and spray coating on an aluminum current collector. The prepared electrode tested for LIB retains 64.5% of initial capacity after 100th cycle. As a reference electrode bare TiO₂-p21 electrode was tested and cycling performance was studied. The cell retains only retains 19.3%. This implies that the addition of rGO and the way that incorporating rGO into the electrodes matters for the performance of the cell.

Hydrothermal temperature and NaOH concentration for the growth of TiO₂ nanobelts were studied, and the optimum temperature was obtained as 175 °C with NaOH concentration of 10 mol dm⁻³. The length of synthesized TiO₂ nanobelts laid between ~15 μm to 410 nm with an average width of ~25 nm to ~64 nm. To increase the structural re-arrangement, TiO₂ was calcined at 450 °C to obtain the anatase phase. However, a small quantity of TiO₂ remained at TiO₂ (B) phase, and it was identified after performing the CV due to appearing of three peaks (S1, S2 and A). This heterostructure TiO₂ was combined with GO using the coagulation technique, and TiO₂ nanobelt/rGO composite was synthesized by reducing with L-AA. The fabricated electrode was tested for LIB anode. As a reference electrode, TiO₂ nanobelt without rGO was also tested. TiO₂ nanobelt electrode retains 67.7% of its initial capacity after the 100th charge-discharge cycle and from the TiO₂-p21 study, it only remained 19.3% of the initial capacity. This concludes that the addition of 2D materials will enhance

the performance of the battery due to low particle agglomerations and hence remain constant surface area while lithium-ions charging and discharging. After the addition of rGO to TiO₂ nanobelts, the capacity retention was improved.

Nb₂O₅/rGO microcomposite was synthesized, following an efficient and novel chemical route using commercially available Nb₂O₅. PXRD analysis confirmed that raw Nb₂O₅ consist of both monoclinic (39%) and orthorhombic (61%) phases. When the temperature of the system increased up to 90 °C, the percentage of Nb₂O₅ phases significantly changed from 39% to 96.2% in the monoclinic phase and 61.0% to 3.8% in the orthorhombic phase. The actual Nb₂O₅ weight (86.7%) in the composite was calculated using TGA analysis before calculating specific capacity of battery. The microcomposite electrode showed 227.3 mA h g⁻¹ of charge capacity and 194.5 mA h g⁻¹ of discharge capacity with 85.8% coulombic efficiency in the 1st cycle under 50 mA g⁻¹. The microcomposite delivers a discharge capacity of 147.3 mA h g⁻¹ after 200 cycles with a 100% coulombic efficiency and 75.7% capacity retention under the same discharge current. The signature rate performance shows that, under 5000 mA g⁻¹ discharge current, Nb₂O₅/rGO electrode retains 27.9% of capacity while Nb₂O₅ retains 3.5%, compared to the discharge current of 50 mA g⁻¹.

Nb₂O₅ microcolumns were synthesized using a facile hydrothermal method by controlling the reaction time. When the reaction was performed for 30 minutes, the morphology of Nb₂O₅ changed to microcolumns, and a further increment of time (60 min.) resulted in transforming Nb₂O₅ microcolumns into microcolumns/cubes mixture. When the reaction further proceeded (180 min.) resulted in the formation of Nb₂O₅ microcubes. To fabricate the Nb₂O₅ microcolumn anode, six electrodes were prepared with different weights ratios of Nb₂O₅ and rGO. Electrochemical impedance spectroscopy data confirmed that the electrode, which consists of 65% Nb₂O₅ (or 25% rGO) has the lowest charge transfer resistance (39.67 Ω) corresponds to the highest capacity retention of 91.1% and 163.5 mA h g⁻¹ of discharge capacity after 100 cycles under a current density of 25 mA g⁻¹. Also, it delivers a capacity of 155.7 mA h g⁻¹ under 1C rate.

IV.CONCLUSIONS

This research provides three significant contributions to the field of electrochemical storage devices. These results show that the use of transition metal oxides with graphene composites (TiO₂/rGO and Nb₂O₅/rGO) as an anode material in LIBs for reversible Li⁺ storage is successful. It shows better characteristics for future applications as an anode material in high-power LIBs.

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INSTITUTION WHERE RESEARCH WAS CARRIED OUT

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PERFORMANCE ENHANCEMENT AND COST REDUCTION OF DYE-SENSITIZED SOLAR CELLS BY DEVELOPING NOVEL ELECTROLYTES AND CARBONACEOUS COUNTER ELECTRODES

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I. INTRODUCTION

It is crucial to conduct research on photoelectrochemical (PEC) energy conversion devices to address the rising demand for energy and the dwindling supply of nonrenewable energy sources. Due to their reduced manufacturing cost and ease of fabrication, dye-sensitized solar cells (DSSCs) are a prospective alternative to conventional silicon-based solar cells. The electrolyte is responsible for electron transport between the dye and electrodes, which is vital to the performance of DSSCs. In DSSCs, organic solvent-derived liquid electrolytes are prevalent, and their conductivity and other properties are highly advantageous for device performance.

This research study presents significant advancements in the field of DSSCs by exploring innovative approaches which are discussed in three main chapters. Firstly, a novel liquid electrolyte system is developed, capitalizing the binary cation effect, to enhance the efficiency of DSSCs. The binary salt electrolyte, consisting of LiI and tetraethylammonium iodide (Hex₄NI), demonstrates promising results. The room

temperature ionic conductivity of the binary electrolyte is 11.44 mS cm⁻¹, while the DSSCs energy conversion efficiency reaches 8.37% under an irradiation intensity of 1000 W m⁻². Impressively, the efficiency enhances to 10.57% at a reduced light intensity of 400 W m⁻².

Next, a new gel polymer electrolyte is developed for DSSCs using polyacrylonitrile polymer, Hex₄NI, KI salts, and performance enhancers. By investigating the effects of varying mass fractions of KI, Hex₄NI, and performance enhancers, the temperature dependence of the conductivity, charge transport behavior, and dielectric polarization of the electrolytes are studied. The highest room temperature conductivity of 3.74 mS cm⁻¹ is shown by the sample containing KI only. However, the DSSC employing a mixed salt electrolyte (75 wt.% KI and 25 wt.% Hex₄NI) demonstrates the highest efficiency of 7.36%, highlighting the suitability of the KI and Hex₄NI salt combination to fabricate highly efficient quasi-solid state DSSCs.

In addition, in the pursuit of replacing platinum counter electrodes, low-cost carbon-based composite electrodes are developed. The electrode containing 70 wt.% graphite and 30 wt.% TiO₂ exhibits a conductivity of 1.30 S cm⁻¹ and sheet resistance of 54.84 Ω cm². The optimum DSSC efficiency is achieved with the 80% graphite and 20% TiO₂ counter electrode, striking a balance between charge carrier transport, transfer properties, and catalytic activity. This comprehensive study contributes to the advancement of DSSC technology by introducing two new liquid and gel electrolytes that harness the effect of small and bulk counter ions in iodide ion conducting electrolytes, and by developing low-cost composite counter electrodes for platinum-free DSSCs.

These findings hold promising prospects for the future development of more efficient and cost-effective DSSCs, thus broadening the boundaries of solar energy conversion technology.

II. METHODOLOGY

A. Investigation of the mixed cation effect and the irradiance level dependence on the efficiency of dye-sensitized solar cells

Unless otherwise specified, all chemicals were purchased from Sigma Aldrich with purity levels exceeding 98%. Tetrahexyl-ammonium iodide (Hex_4NI) and LiI were vacuum dried for about 2 h at $\sim 50^\circ\text{C}$ prior to use. The correctly measured weights of compounds, propylene carbonate (PC), ethylene carbonate (EC), 1-butyl-3-methylimidazolium iodide (BMII), 4-tert-butyl pyridine (4TBP) and I_2 were mixed in the relevant order in a closed vial. Before analyzing the liquid electrolyte, the resulting mixture was stirred for approximately one hour with a magnetic stirrer and left at room temperature for approximately forty-eight hours.

Three distinct DSSCs were made by sandwiching three different liquid electrolytes between the TiO_2 multi-layer photo-anodes and the platinum (Pt) counter-electrodes. The electrolyte compositions were selected based on preliminary studies and preoptimizations conducted for gel-based electrolyte systems.

B. Binary counter ion effects and dielectric behavior of iodide ion conducting gel-polymer electrolytes for high-efficiency quasi-solid-state solar cells

Polyacrylonitrile (PAN), tetrahexylammonium iodide, KI, iodine (I_2), PC and EC (all with purity greater than 98%) purchased from Sigma-Aldrich were used as starting materials.

PAN, KI, and Hex_4NI were vacuum dried at 60°C for 24 h prior to use. Weights of EC (1.6604 g), PC (1.5404 g), 4TBP (0.0868 g) and BMII (0.0504 g) were kept unchanged in all the electrolyte samples. The relative weights of KI and Hex_4NI were varied, keeping their total weight constant at 0.37 g. The weight of iodine (I_2) was 0.384 g, which is about one-tenth of the total mole amount of the iodide from the two salts. Appropriately weighed quantities of EC, PC, 4TBP, BMII, KI and Hex_4NI were mixed under the continuous stirring at room temperature for ~ 2 h until the entire amount of salts dissolves. Then, after adding 0.4 g of PAN, the mixture was stirred again for about 30 min. Then the mixture was heated to about 80°C together with continued stirring for about another 15 min. The resulting mixture was allowed to cool down to ambient temperature. Finally, I_2 chips (384 mg) were added and again continuously stirred overnight in a closed bottle to obtain a homogeneous gel-electrolyte. This procedure was repeated for all the electrolyte compositions. Three electrolyte samples were prepared and tested for each composition with most reproducible results were selected for the analysis.

C. Preliminary optimization of low-cost quasi solid-state dye sensitized solar cells with multilayer photoanodes and graphite based counter electrodes

A series of composite counter electrodes were prepared using a mixture of graphite and TiO_2 powder. The relevant weights of TiO_2 nanoparticles (particle size 21 nm) received from Evonik (AEROXIDE® TiO_2 P 25) and commercial Graphite powder (BDH Chemicals Ltd Poole England – Prod 26109) were used. The compounds were mixed in 4 ml of HNO_3 for about 30 minutes under rigorous stirring. The slurry obtained was then coated on the conducting side of pre-

cleaned fluorine-doped tin oxide (FTO) (Solaronix SA) conductive substrate, using the doctor blade technique. After 24 h of air-drying, the graphite coated FTOs were annealed at 450°C for 30 min and then used as the counter electrode.

III. RESULTS AND DISCUSSION

A novel organic electrolyte is synthesized using EC and PC as co-solvents, LiI, and tetra-hexylammonium iodide as salts, 4TBP and BMII as performance enhancers. According to the temperature dependence of conductivity, the electrolyte displays non-Arrhenius behavior but obeys the VTF relation. The single salt electrolyte system containing Li^+ shows the highest conductivity at all temperatures measured. The new binary salt electrolyte prepared for DSSCs possesses a conductivity value of 11.44 mS cm^{-1} at 30°C . The conductivity of Li^+ single salt electrolyte shows 11.36 % enhancement compared to that of binary salt system, at 30°C . The combined use of the LiI and Hex_4NI salts demonstrates the impact of binary cation on efficiency when compared to single salt systems, exhibiting an impressively high energy conversion efficiency. Under 1000 W m^{-2} illumination, the energy conversion efficiency of the DSSCs using this binary salt electrolyte is 8.37% which is an efficiency enhancement of 86.90% and 76.32% compared to Hex_4NI and LiI based single salt counterparts, respectively. The effectiveness of DSSCs in varying lighting situations is examined in this study. When the light intensity is reduced to 400 W m^{-2} , the efficiency is as high as 10.57%. Overall, using a binary salt system in the electrolyte of DSSCs is a promising way to increase efficiency in DSSCs.

A new set of GPEs based on the PAN polymer host, which is suitable to fabricate highly efficient quasi-solid-state DSSCs harnessing the mixed cation effect, were investigated. The new GPEs are composed of PC and EC co-solvents, Hex_4NI and KI salts and 4-tertbutylpyridine and 1-butyl-3-methylimidazolium iodide performance enhancers. The analysis of ionic conductivity in electrolytes reveals that the temperature dependence of the electrolyte exhibits VTF (non-Arrhenius) behavior. The ionic conductivity in the electrolyte increases with the increasing mass fraction of KI (decrease of Hex_4NI). The highest conductivities at all the temperatures are given for sample E with 100 wt.% KI and it shows values of 3.74 , 6.07 and 7.86 mS cm^{-1} at 23 , 50 and 70°C , respectively. Frequency dependence of the real and imaginary parts of the AC conductivity is analyzed in order to understand polarization effects on electrical/dielectric properties. The real part of the AC conductivity increases with increasing temperature as well as increasing frequency as a result of an increase in ion transport dynamics. Peak values of the imaginary part of the AC conductivity are visible at about 10 kHz due to charge transport relaxation. A set of DSSCs assembled using N719 dye-sensitized TiO_2 electrodes and the new electrolyte series showed efficiencies higher than 5%.

Highlighting the mixed cation effect, the cells with mixed iodide salts show efficiencies greater than 6%. Consequently, an impressively high energy conversion efficiency of 7.36% is achieved by the quasi-solid- state DSSC prepared with electrolyte containing 75% KI and 25% Hex_4NI . This study suggests that the salt combination KI and Hex_4NI provides a suitable iodide salt mixture for the fabrication of high-efficiency DSSCs.

A series of low-cost counter electrodes were prepared using TiO_2 and graphite for the dye-sensitized solar cells (DSSCs) to replace expensive Pt electrodes. The composition of TiO_2 and graphite electrodes were optimized by analyzing electrical transport properties and incorporating them successfully into quasi-solid state DSSCs. The conductivity of composite having 70 wt.% graphite and 30 wt.% TiO_2 was 1.30 S cm^{-1} and the sheet resistance of the respective electrode was $54.84 \text{ } \text{\AA cm}^{-2}$. With the decrease of graphite content, (i.e. increase of the amount of TiO_2) the sheet resistance of the electrodes decreases initially, and after reaching the minimum for 70% graphite composition, it increases back again. The XRD analysis reveals the drop of crystallinity of graphite in the composite with added TiO_2 as a result of mechanical exfoliation of graphite forming expanded graphite or multilayer graphene films. In addition, when used as a counter electrode, the amount of graphite plays a critical role due to its catalytic activity. However, the utilization of 100% graphene is not desirable due to inefficient film formation and film adhesivity to FTO. This optimum composition ratio was confirmed by using two series of DSSCs having 5 and 6 TiO_2 layers in the photo-anode. Further, it was confirmed that the 6-layer TiO_2 photoanode gives the highest DSSC performance by investigating 7-layer photo-anodes alongside 5 and 6-layer versions. The best DSSC efficiency was exhibited by the counter electrode that contained 80% graphite and 20% TiO_2 . This composition gives optimum output as a result of the trade-off between charge carrier transport and transfer properties and catalytic activity of the electrode. Finally, it can be concluded that graphite/ TiO_2 counter electrodes are suitable to prepare low-cost quasi solid-state DSSCs.

The overall performance of the DSSCs has increased significantly. These findings hold promising prospects for the future development of more efficient and cost-effective DSSCs, thus broadening the boundaries of solar energy conversion technology.

IV. CONCLUSIONS

This research provides three significant contributions to the field of dye-sensitized solar cells. The first significant contribution presented here is the investigation of the mixed cation effect and the irradiance level dependence on the efficiency of dye-sensitized solar cells. It could provide DSSCs with comparable efficiency and high stability to liquid-type electrolytes. The second significant contribution presented here involves the binary counter ion effects and dielectric behavior of iodide ion conducting gel-polymer electrolytes for high- efficiency quasi-solid-state solar cells. This project has convincingly shown that a new set of GPEs based on the PAN polymer host, which is suitable to fabricate highly efficient quasi-solid-state DSSCs harnessing the mixed cation effect, investigation. The third contribution in this thesis involves a preliminary optimization of low-cost quasi solid- state dye sensitized solar cells with multilayer photoanodes and graphite-based counter electrodes. For this purpose, a series of low-cost counter electrodes were prepared using TiO_2 and Graphite for the dye-sensitized solar cells (DSSCs) to replace expensive Pt electrodes. The composition of TiO_2 and graphite electrodes were optimized by analyzing electrical transport properties and incorporating them successfully into quasi-solid state DSSCs. With these optimizations, it was possible to achieve. The best DSSC efficiency was exhibited by the counter electrode that

contained 80% graphite and 20% TiO_2 . It can be further developed through a research and development phase to fabricate practical and prototype solar cells.

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Cu - NITROGEN DOPED GRAPHENE (Cu–N/Gr) NANOCOMPOSITE AS CATHODE CATALYST IN FUEL CELLS – DFT STUDY

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I. INTRODUCTION

Energy security and sustainability are the impetus for modern economics. Transformation of the energy ecosystem requires the search for cost-effective, secure, and sustainable energy technologies to replace incumbent energy sources. Fuel cells contribute well to this transformation as they evidently have their merits, particularly for compactness and zero emissions, such as proton exchange membrane fuel cells (PEMFCs).

Low-temperature fuel cells operate at temperatures below 200 °C and a carbon support catalyst is deployed to improve the oxygen reduction reaction (ORR). Carbon support catalysts are not conducive to high temperature operations, leading to an increased rate of carbon corrosion and, therefore, degrading the carbon support (Antolini 2009). Corrosion of the carbon support will directly affect the catalyst layer, causing the catalyst materials to agglomerate.

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Research on copper is mainly based on a single metal with nitrogen atoms as dopants (Bhatt, Lee et al. 2017, Xiao and Zhang 2020), which gives good ORR performance, and spontaneous ORR makes them more favorable for catalysts. A single metal Cu-N₂/Gr structure shows that it has a higher probability of following two-electron pathways to generate H₂O₂. In addition, there was no indication of the cathode potential of the Cu-nitrogen doped catalyst. A single structure of Cu-N₂/Gr cannot describe the H₂O₂ generation capability, or the cell potential of the Cu-nitrogen doped catalyst. Therefore, other structures, such as dual-metal structures, should be considered.

Research shows that Fe-based non-PGM catalysts Fe₂-N₆/Gr and Fe₂-N₈/Gr have good formation energies and ORR performance (Zhang, Zhou et al. 2020, Chen, Sun et al. 2022). Since Cu has shown better ORR performance steps than Fe (Nørskov, 2004), the Cu₂-N₆/Gr and Cu₂-N₈/Gr structures should have considerably better ORR performance than the single metal Cu-nitrogen doped catalyst or Fe-based non-PGM catalysts. To prove this hypothesis a DFT study was performed in an acidic medium with different potentials at 298.15 K to determine the ORR mechanism and stability of Cu₂-N₆/Gr, Cu₂-N₈/Gr, and Cu-N₄/Gr electrocatalysts. From these calculations the following were predicted.

- i. Structural stability of structures.
- ii. Binding ability of ORR steps.
- iii. Break-free ability of H₂O molecule from catalyst surface.
- iv. Evidence of H₂O₂ generation.
- v. Maximum cell potential.

II. METHODOLOGY

DFT calculations were performed with Gaussian 09w software using the B3LYP/3-21G basis set with non-periodic and non-dispersion interaction. Defect structure is visualized using GaussView 6.0. All atoms in every structure were relaxed by optimization. A common single pristine graphene layer was developed, and it is used to develop other structures (Cu₂-N₆/Gr, Cu₂-N₈/Gr, and Cu-N₄/Gr) to investigate ORR considering the defect structure at the catalyst surface. All structures were developed to contain only pyridinic nitrogen. After optimization, zero imaginary frequency for dual atom structures and one (1) imaginary frequency for the Cu-N₄/Gr

structure. Additionally, the optimized charges of all three catalysts were zero and singlet spin for all dual atom structures. The molecules used in the ORR steps were individually optimized. Bond lengths and bond angles were measured and compared with those of previous research.

Catalyst structures based on Cu confirmed their stability with the cathode potential. Stability was inspected using the formation energy (ΔE),

$$\Delta E = E_{graphene+(M_a-N_b)} + y\mu_C - (E_{graphene} + x\mu_N + E_M)$$

Here, $E_{graphene+(M_a-N_b)}$ is energy of optimized graphene layer with Cu-N defect. The 'a' and 'b' are positive integers defining the selected Cu-N defects ($a=1,2$ and $b=4,6,8$). M and N are Cu and nitrogen, respectively. The μ_C and μ_N are chemical potential of carbon defined as total energy per carbon atom for defect-free graphene, and the chemical potential of nitrogen defined as half of the total energy of N_2 molecule, respectively. x and y are the number of nitrogen atoms added and the carbon atoms removed during the defect formation, respectively. $E_{graphene}$ is the energy of optimized pristine graphene layer. E_M is the total energy of M^{n+} defined as,

$$E(M^{n+}) = E(M) - neU$$

where, $E(M)$ is the total energy of isolated M (M= Cu) in the gas phase and, n, e, U are the number of electron transfer (+2), electron charge, and external potential in order.

Binding energies (BE) were calculated from the ORR steps in an acidic medium, as defined by eq.,

$$BE = E_{defect+molecule} - (E_{defect} + E_{molecule})$$

Here, $E_{defect+molecule}$ is the total energy of molecules adsorbed by defect graphene. E_{defect} is the total energy of defect graphene configuration, and $E_{molecule}$ is isolated molecule species (O_2 , O, H_2O , OOH , OH, H_2O_2). Negative signed binding energies ($E_{defect+molecule} < (E_{defect} + E_{molecule})$) indicate it is more favorable for molecules to be attached to the defect configuration. The formation of H_2O_2 during ORR is also considered.

The free energies were calculated for each ORR step of all defects configurations as defined by eq.,

$$\Delta G = \Delta E + \Delta ZPE - T\Delta S + \Delta G_U + \Delta G_{pH} + \Delta G_{field}$$

ΔE is the energy from the DFT calculation to the relevant reaction step, and ΔZPE is the correction of zero-point energy is obtained from the NIST database and DFT calculations. T and S are the absolute temperature and entropy, respectively. Here, $T = 298.15$ K, and the entropy value was obtained from the NIST database. $\Delta G_U = -eU$ where U and e are the electrode potential and the charge transferred, respectively. $\Delta G_{pH} = K_B T \times \ln 10 \times pH$,

where K_B is Boltzmann's constant and $T = 298.15$ K. ΔG_{field} is a contribution of the interaction of adsorbate with the local electric field in the electric double layer formed in the vicinity of the cathode, which is negligible according to previous studies. Corrections of zero-point energy and entropy values were obtained from frequency calculations for the molecule absorbed by defect graphene. The free energy vs. reaction coordinate graphs were drawn with different potentials (U) for each structure.

The Mulliken charge population was applied to molecules as defined by eq.,

$$\Delta Q_X = Q_{\text{after}} - Q_{\text{before}}$$

The Q_{after} and Q_{before} are charges of the molecule (X) after and before adsorption, respectively.

The energy gap (E_g), chemical hardness(η), chemical potential (μ), and electrophilicity index (ω) were calculated using the Koopman's principle for optimized structures as defined by the eq.,

$$\begin{aligned} E_g &= E_{LUMO} - E_{HOMO} \\ \eta &= I - A \\ \mu &= -\frac{(I+A)}{2} \\ \omega &= \frac{\mu^2}{2\eta} \end{aligned}$$

Here, I and A are the ionization potential ($\cong -E_{HOMO}$) and the electron affinity ($\cong -E_{LUMO}$), respectively.

III. RESULTS AND DISCUSSION

The formation energies of the Cu_2-N_6/Gr , Cu_2-N_8/Gr , and $Cu-N_4/Gr$ structures were -23.60, -23.49 and -13.22 eV in order at zero potential ($U = 0$) under open circuit conditions. Negative formation energies are favorable for the formation of stable structures, and the formation energies of all the structures studied remain negative between the potential range of 0 to 5.87 V. The critical cell potential of negative formation energies was observed at 5.89, 5.87, and 6.60 V for Cu_2-N_6/Gr , Cu_2-N_8/Gr , and $Cu-N_4/Gr$, respectively. The structures forming stability follow the increasing order of $Cu-N_4/Gr < Cu_2-N_8/Gr < Cu_2-N_6/Gr$, as indicated by the external potential, U , at zero.

As a first ORR step, O_2 binding to the defect shown in this study is significantly high, except for the $Cu-N_4/Gr$ structure in relation to $Cu-N_2/Gr$. Structure-wise $Cu-N_2/Gr$ has a higher O_2 binding energy than $Cu-N_4/Gr$, also, Cu_2-N_6/Gr has a higher binding energy compared to the Cu_2-N_8/Gr structure. This indicates that adding more nitrogen atoms to Cu did not create a good O_2 adsorbent defect. The efficiency of the O_2 adsorbent in decreasing order is as follows, $Cu_2-N_6/Gr > Cu_2-N_8/Gr > Cu-N_4/Gr$. The OH and OOH binding energies of structures also signify the above N atoms and the idea of a stable defect.

ORR pathways for Cu_2-N_6/Gr showed two possibilities by optimization. These are named Cu_2-N_6/Gr (01) and Cu_2-N_6/Gr (02). The difference in free energy (ΔG) values

indicate that the Cu₂-N₆/Gr structure favors both ORR pathways.

The H₂O binding strength follows a decreasing order with respect to the defect structures: Cu₂-N₆/Gr > Cu-N₄/Gr > Cu₂-N₈/Gr. Based on the results of this study, it has been confirmed that the attachment of H₂O to the Cu atom on the Cu₂-N₈/Gr defect structure is weakest, as evidenced by the 2.20 Å Cu-O bond length, when compared to the other defect structures investigated. On the other hand, Cu₂-N₆/Gr is shown to have a high H₂O adsorbent possibility but also to have a better adsorption ability for O₂ than H₂O. The binding energies indicate that Cu-N₄/Gr has less ability to adsorb H₂O when compared to Cu₂-N₆/Gr. The bond length to H₂O is similar for both defects. The binding energies of *OH, *OOH and *O have significantly higher values, indicating that all three defects could be considered as possible stable active sites for ORR.

The Mulliken charge population (ΔQ_x) was applied to both O atoms of the O₂ intermediate for all structures. The decreasing order of the charge population bonding strength is Cu₂-N₆/Gr > Cu₂-N₈/Gr > Cu-N₄/Gr, suggesting that that Cu₂-N₆/Gr has the highest O₂ adsorption ability. For H₂O, decreasing order as Cu-N₄/Gr > Cu₂-N₈/Gr > Cu₂-N₆/Gr (01) > Cu₂-N₆/Gr (02). Only Cu-N₄/Gr and Cu₂-N₈/Gr are compared with the above H₂O adsorbent strength decreasing order explained by the binding energy. The difference in H₂O adsorbent strength order between the Mulliken charge population and the binding energy may be due to the O atom drawing electrons from H atoms of H₂O, altering the Mulliken charge values of the O atom. Based on the Mulliken charges of Cu₂-N₆/Gr, it has the weakest bond strength on H₂O, allowing H₂O to break free from the catalyst easily.

Based on the chemical hardness, chemical potential and electrophilicity calculations, the Cu₂-N₈/Gr shows a higher stability and favors ORR than Cu₂-N₆/Gr.

By free energy calculations, The Cu₂-N₆/Gr motif is not recognized as a spontaneous catalyst. Therefore, considering these three motifs, only Cu₂-N₈/Gr and Cu-N₄/Gr show promising ORR ability. The maximum cell potentials for spontaneous reactions are shown to be 0.49 and 0.28 V for Cu-N₄/Gr and Cu₂-N₈/Gr, respectively. The Cu-N₄/Gr defect structure has a greater possibility of forming H₂O₂, as indicated in ORR steps by optimization. The free energy diagram shows that the formation of H₂O₂ was spontaneous within the range of 0 - 0.49 V.

IV. CONCLUSIONS

Computational calculations predicted the Cu₂-N₈/Gr and Cu-N₄/Gr motifs, showing promising spontaneous ORR with maximum cell potential of 0.28 and 0.49 V under standard conditions. The Cu₂-N₆/Gr motif is not favorable for spontaneous ORR. From the formation energy calculations, Cu₂-N₈/Gr, Cu-N₄/Gr, and Cu₂-N₆/Gr are stable for potential range (U) 0 - 5.87, 0 - 6.6, and 0 - 5.89 V, respectively. The maximum cell potentials of Cu₂-N₈/Gr and Cu-N₄/Gr are within the potential range of formation energy, showing

structural stability. Based on the chemical hardness, chemical potential, electrophilicity, and Mulliken charge calculations, the Cu₂-N₈/Gr shows a higher stability and favors ORR than Cu₂-N₆/Gr. Cu₂-N₆/Gr is more favorable for ORR than Cu-N₄/Gr by binding energy values. The Cu₂-N₆/Gr motif is not recognized as a spontaneous catalyst by free energy calculations. Therefore, considering these three motifs, only Cu₂-N₈/Gr and Cu-N₄/Gr show promising ORR ability. Consequently, the possibility of cell voltage solely depends on Cu₂-N₈/Gr could occur due to its high stability. According to DFT optimization, H₂O₂ is generated only from Cu-N₄/Gr. The other structures follow four-electron transfer pathways to generate high electron density. The Cu-Nitrogen doped non-PGM catalyst with the considered motifs has a relatively high current density even with low cell voltage when comparing to Pt-like catalysts. The amount of H₂O₂ generated by Cu-N₄/Gr could be limited by its low stability. Previous studies have also shown that the Cu-N₂/Gr motif has a high probability of generating H₂O₂ but the -5.68 eV formation energy indicates that it has lower stability than Cu-N₄/Gr. In this study, a novel Cu₂-N₈/Gr catalyst is a good candidate for fuel cell application as it has good cell potential and non-H₂O₂ formation.

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INSTITUTION WHERE RESEARCH WAS CARRIED OUT

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HIGH SURFACE CONDUCTING, LOW TRANSPARENT FLUORINE-DOPED TIN OXIDE THIN FILMS WITH LIGHT SCATTERING MORPHOLOGY FOR DYE-SENSITIZED SOLAR CELL APPLICATIONS

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I. INTRODUCTION

Transparent conductive oxide films are one of the main components of a multitude of electrochemical devices, such as dye solar cells (DSCs) and supercapacitors. Fluorine-doped tin oxide (FTO) films are the most widely used substrates due to their inexpensive preparation process and higher temperature tolerance. As a rule of thumb, FTO films with 80% transmittance and sheet resistance of $10 \Omega \square^{-1}$ are usually chosen for solar cell fabrication. However, there is a fundamental trade-off between optical transmittance and electrical sheet resistance, which inhibits the simultaneous increase of the transmittance and the surface conductivity. The possibility of reducing sheet resistance below $10 \Omega \square^{-1}$ is affected by the reducing transmittance with increase in film thickness. Besides there are several attempts to enhance the efficiency of DSCs by introducing an additional light scattering layer to the photoelectrode which fabricated on conventional FTO substrates. The question is, is it possible to reduce sheet resistance of FTO films while increase in the light scattering ability and use them for improvement of performance of DSCs? In addition, the literature shows that most of the DSCs fabricated for research purposes utilized commercially available FTO substrates. This restricts the possible variation of some parameters crucial in optimizing cell performance. This work introduces a facile and low-cost method of preparing FTO films and modifying necessary parameters enabling the utilization of tailor-made FTO films in related applications.

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The aims and objectives of the study were as follows:

Aims:

- i. To study the effect of surface morphology and film thickness for the optical and electrical properties of FTO thin films.
- ii. To study the effect of sheet resistance, conductivity, surface morphology, transmittance and light scattering properties of FTO substrate for the performance of DSCs.
- iii. To study the effect of the solvent type used in the spray pyrolysis method, for the optical and electrical properties of FTO thin films.
- iv. To compare the performance of the DSCs which are based on different FTO films prepared by different precursor solvents.
- v. To enhance the performance and energy conversion efficiency of DSCs.

Objectives:

- i. Determine the most suitable spray pyrolysis parameter values by referring literature and conducting several test runs, to obtain FTO films with better conducting property and light scattering morphology.
- ii. Preparation of three FTO series by change in the film thickness and precursor solvent type.
- iii. Characterization of electrical, optical, structural and morphological properties of prepared FTO series and commercially available FTO films.
- iv. Fabrication of three DSC series from prepared FTO samples and commercially available FTO substrates.
- v. Characterization of prepared DSC series to analyse and compare the cell performances.

II. METHODOLOGY

A. Preparation of FTO films

Three series of FTO films were prepared using three different solvents, which were methanol (Sigma-Aldrich, $\geq 99.8\%$), absolute ethanol (Sigma-Aldrich, 99%), 2-propanol (VWR analytical grade). First, 9.01 g of

$\text{SnCl}_4 \cdot 5\text{H}_2\text{O}$ (tin(iv) chloride pentahydrate; Sigma-Aldrich 99.995%) was added to 400.0 cm^3 of methanol and the mixture was sonicated for 15 minutes to obtain a clear solution with the help of ultra-sonication. Then, 2.4 g of saturated aqueous solution of NH_4F (Ammonium fluoride; Sigma-Aldrich 99%) was added to the above solution and the mixture was sonicated again for further 15 minutes. The resulted, optically transparent solution was used to fabricate the first series of FTO films. Similar procedure was repeated for the other two solvents except, 0.01 M oxalic acid~ 40 ml was added to the 2-propanol solvent to obtain a transparent precursor.

Commercially available, soda-lime glass plates ($1 \times 25 \times 25$ mm) were used as substrates for the deposition of FTO. First, the glass plates were cleaned by sonication in a mixture of ethanol and acetone (1:1 v/v) for 10 minutes, and were then dried in a hot air flow, and make sure there is no any contamination on the glass plates.

Sequential nebulizer spray pyrolysis technique was used to prepare FTO films. A custom-made spray gun, prepared by using two glass droppers, was utilized to spray the precursor solution onto the glass substrate. The glass substrates were placed on a hot plate and spraying procedure was done inside a fume cupboard to avoid toxic gases. During the spraying, temperature of the glass subtract was maintained at 500 °C and the distance between the hot plate and spray gun was maintained 10 cm. The spraying was conducted along longitudinal and lateral directions about 10 s, so that it covers the entire substrate and then, waited about 50 s to sinter. The same procedure was repeated until finish the 40 ml of solution. After the spray coating, the sample was kept at 500 °C about 30 min. This process, spraying of 40 ml of the solution and subsequent heating to 500 °C, is considered as preparation of one-layer or one spray cycle. The procedure of deposition of FTO film was same for all three types of precursors and 7 samples were prepared from each precursor solvent by change in the number of spray cycles from 1 to 7. Three series of FTO samples prepared by using, methanol solvent (namely M1, M2,..., M7 or M-FTO series), ethanol solvent (namely E1, E2,..., E7 or E-FTO series) and 2-propanol solvent (namely P1, P2,..., P7 or P-FTO series).

B. Preparation of DSCs

Among above prepared FTO samples, first six samples of each FTO series and a commercially available FTO substrate (CFTO) (SOLARONIX TCO10-10, Thickness: 1.1 mm, Sheet resistance: $10 \Omega \square^{-1}$, Transmittance: >80% from 500 to 800 nm) were used to prepare photo-electrodes (size 1×2 cm) for DSC assembly. The preparation of photo-electrodes, electrolytes, counter-electrodes, and DSCs was done according to an already published method (Nishshank, G. B et al., 2020). The electrolyte contained polyethylene oxide (PEO), ethylene carbonate (EC), propylene carbonate (PC) 1-butyl-3-methylimidazolium iodide (BMII), and 4-tert-butylpyridine (4TBP), tetrahexylammonium iodide (Hex₄NI), lithium iodide and iodine. The electrolyte was prepared using the stoichiometric composition of $\text{PEO}_{10}\text{EC}_{40}\text{PC}_{40}\text{BMII}_{0.25}\text{4TBP}_{0.85}\text{Hex}_4\text{NI}_{0.8}\text{LiI}_{1.2}\text{I}_{2(0.2)}$, following the already published method. The photo-electrodes were prepared by spin coating two different particle sizes (13 nm and 21 nm) of TiO_2 nano-particles on the different FTO substrates. Three series of DSCs (namely M-DSC, E-DSC and P-DSC series) were assembled by sandwiching the gel

polymer electrolyte between a Pt counter electrode and dye-sensitized photo-electrode, which were fabricated on M-, E- and P-FTO substrates. In addition a reference DSC was prepared by using CFTO base photo-electrode. The final configuration of each DSC was; [glass/ FTO/ TiO_2 / N719 dye/ gel polymer electrolyte/ Pt counter-electrode].

C. Characterization of the FTO series

The sheet resistance of each sample and the commercial FTO were measured using a four-probe setup via a VK-PA-25 PV power analyzer. The measurements were taken along the two perpendicular directions parallel to the edges of the sample. The transmittance of each FTO sample was measured using a double-beam UV-visible spectrophotometer (UV-1800 series). The glass substrate was used as the reference, and the spectrum was obtained within the 300-900 nm wavelength range. For comparison, the transmittance spectra of a commercial FTO glass (CFTO) and the soda-lime substrate glass were obtained within the same wavelength range. XRD spectra of FTO samples with layers 2, 4, 6 in each FTO series and CFTO were obtained using $\text{Cu}-\text{k}_\alpha=1.5405\text{\AA}$ radiation of the Rigaku Ultima IV X-Ray Diffractometer (KYOWAGLAS-XA) within the 20-90° angular range. Scanning electron microscopic (SEM) images of the surface and cross sections of the prepared FTO samples and CFTO reference were taken by using EVO LS 15 scanning electron microscope.

D. Characterization of the DSC series

Fabricated solar cells were irradiated with light of 1000 W m^{-2} (AM 1.5) by a PEC-LO1 solar simulator, and the current-voltage (*I-V*) characteristics of the cells were obtained. The active area of a cell was 19 mm^2 . Current-voltage data were measured for 3 h (at 10 min steps) under continuous irradiation. Nyquist plots of the fabricated DSCs were obtained using a Metrohm Autolab potentiostat by keeping the direct current (DC) potential set at maximum open circuit voltage (V_{oc}) and setting the alternative current (AC) amplitude as 0.01 V in a frequency range from 0.01 to 106 Hz.

III. RESULTS AND DISCUSSION

The sheet resistance (R_{sh}) decreased gradually with the number of spray cycles and reached a plateau. Conductivity of the isopropanol-based FTO series is the highest while methanol-based series is the lowest. The reason may be variation of the boiling points of the solvents. An increasing trend can be seen in conductivity with number of spray cycles. This may be due to the increase in crystallinity and the particle size of the thin films.

Transmittance of FTO with single spray cycle comparable with CFTO. The E-FTO relatively high transmittance compared to other two series. The reason for this may be the moderate, boiling temperature and the dipole moment of the ethanol compared to other two solvents, which results for smooth surface with small particle sizes as we can observed from the SEM morphology.

No significant variation of the bandgap of the thin films with number of spray cycles or solvent type.

Average optical bandgap value of the M-, E- and P-FTO series were 3.76 ± 0.03 eV, 3.76 ± 0.02 eV and 3.77 ± 0.02 eV respectively. They are bit lower than the bandgap value of the

CFTO reference (3.87 eV). No considerable differences between the structure of prepared FTO and the CFTO. However, E2 and E4 samples show very low intense, additional crystallographic plane which is relevant to tetra tin oxide hexafluoride phase. M-FTO series shows the lowest intensity in (200) crystal plane while the intensity of (110) and (101) crystal planes are relatively high.

SEM images show clear change in morphology with solvent type. M- and P-FTO samples shows polygonal shaped particles with sharp edges while the E-FTO samples have more rounded shapes. Particle sizes increased with number of spray cycles.

DSC fabricated with the P3-FTO has resulted in the best cell efficiency of 5.14%. It is higher than CFTO based DSC (4.74%). Efficiency shows increasing trend with number of spray cycles and reached to a maximum value, then decreased gradually. Increase in trend may be due to; increase in surface conductance, increase in light scattering. Reason for the decrease in trend may be energy lose while scattering, indirect absorption of light from the FTO films, increase in light attenuation with thickness.

IV. CONCLUSIONS

It is possible to reduce the sheet resistance of FTO films up to $\sim 1 \Omega \square^{-1}$ in all three FTO series. However, the bulk conductivity of the films depends on the solvent type. The highest conductivity shown by the P-FTO series, which prepared by the solvent with relatively high boiling point. In addition, conductivity shows increase in trend with increase in the number of spray cycles. In terms of optical properties, highest transmittance showed by the samples in the E-FTO series which have low particles sizes and smooth surface compared to other two FTO series. The reason for this may be the intermediate values of boiling point and solubility. However, there is no significant variation of the optical band gap with neither solvent type nor spray cycles. There is no significant difference in term of structure except the peak intensities, where the most preferable crystal plane growth varies with solvent type. Surface morphology of the FTO films have been affected by the precursor solvent type. As evident from the SEM images the particle size and the shape varies with solvent type and number of spray cycles.

In all three DSC series the energy conversion efficiency shows increase in trend with number of spray cycles deposited in the FTO utilized for the photo-electrode and reached to a maximum value, then decreased gradually. The increase in trend may be due to increase in surface conductance (decrease in R_{sh}), bulk conductivity and increase in light scattering ability of the FTO film. The reason for the decrease in trend may be the energy lose while scattering of light and indirect absorption of light from the FTO films and increase in light attenuation with thickness. Better energy conversion efficiencies can be obtained with low-transparency FTO films having low sheet resistance with light scattering morphology compared to conventional, highly transparent films. For example, DSCs prepared using the P- and M-FTO substrates having very low 7.9, 7.0, 4.5, 8.1 and 4.2% transmittances have shown 4.66, 4.35, 3.17, 3.64 and 3.28% energy conversion efficiencies, respectively. The highest recorded energy conversion efficiency of 5.14% was obtained for the P3-FTO film with 34.2% transmittance and $3.27 \Omega \square^{-1}$ sheet resistance, and it was considerably higher than that of standard

commercial FTO films (4.74%) with ~83% transmittance and $10 \Omega \square^{-1}$ sheet resistance. The results indicate that, instead of molding an additional light scattering layer to the photo-electrode, translucent FTO films with carefully engineered light scattering properties can be used in fabricating dye sensitized solar cells with superior efficiency. The out of box thinking of neglecting the transmittance and focusing on the translucent properties of the FTO films opening the way to surpass the barrier of enhancing the surface conductance of conventional FTO for the solar cell application.

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PLANT SCIENCE

PLANT HORMONAL REGULATION ON PHYSIOLOGICAL CHANGES IN TEA (*Camellia sinensis* L.) DURING DROUGHT STRESS

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I. INTRODUCTION

Drought stress is one of the most significant environmental factors affecting plant growth, development, and productivity. According to the literature, phytohormones such as abscisic acid (ABA), auxin, cytokinin, ethylene, gibberellic acid (GA), jasmonates, and salicylic acid (SA) play key roles in regulating plant responses to abiotic stress conditions (Verma *et al.*, 2016). Consequently, understanding plant hormonal regulation is crucial for developing crop management practices that mitigate the negative effects of drought stress. While considerable attention has been given to the exogenous application of phytohormones in various crops to reduce the adverse effects of drought, limited information is available on tea. Furthermore, the hormonal regulation of tea under drought stress remains poorly understood, particularly regarding its effects on gas exchange, water balance, and osmolyte accumulation. Therefore, this study was designed to achieve the following objectives.

The main objective of the current study is to investigate the impact of the exogenous application of selected hormones on the physiological and biochemical responses of tea plants under drought stress conditions.

The following specific goals were set for the study to accomplish the main goal:

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- i. To evaluate the potential of exogenous hormone application in improving productivity and survival under short-term drought conditions.
- ii. To investigate the effects of plant hormones on the antioxidant defense mechanisms during drought stress.
- iii. To determine the economic feasibility of foliar application of different plant hormones to mitigate the adverse effects of drought stress in tea.

II. METHODOLOGY

A. Glasshouse experiments

Glasshouse experiments were conducted at the Tea Research Institute of Sri Lanka using one-year-old cultivars, namely TRI 2025 (drought-tolerant) and TRI 2023 (drought-susceptible), arranged in a Randomized Complete Block Design (RCBD) with 2 blocks and 24 replicate plants per cultivar per treatment. The plants were subjected to a drying cycle by withholding water. Upon reaching moderate moisture stress, the plants were foliar-sprayed with abscisic acid (ABA), salicylic acid (SA), and gibberellic acid (GA) individually at various concentrations [0 (water-spray – WS), 50, 100, 150, and 200 mg L⁻¹], along with well-watered (WW) and no-spray (NS) treatments.

The effective concentration identified from the glasshouse study was further tested under field conditions at Bearwell Estate and St. Coombs Estate, Talawakelle, using immature and mature tea plants, respectively.

B. Field Experiments

These experiments were conducted at Field No. 08NC, Fairfield Division, Bearwell Estate, Talawakelle (agro-ecological region WU2) using the three-year-old cultivar TRI 4078, and at Field No. 10, Lower Division, St. Coombs Estate, Talawakelle (agro-ecological region WU2) using a 23-year-old mixture of cultivars. The experiments were arranged in a Randomized Complete Block Design (RCBD) with 3 blocks. When the plants reached moderate moisture stress, they were foliar-sprayed with ABA (100 mg L⁻¹), GA (50 mg L⁻¹), and SA (150 mg L⁻¹), based on the results of previous glasshouse studies, along with water-spray (WS) and no-spray (NS) treatments.

Physiological and biochemical parameters were measured, along with soil moisture content, in both glasshouse and field experiments at specified intervals after applying the treatments. A portable photosynthesis system (model: LI-6400XT, Li-Cor Inc., USA) was used to determine the net photosynthetic rate, stomatal conductance, and transpiration rate. Dark respiration was also measured in another set of leaves by covering them with aluminum foil for 30 minutes prior to the gas exchange measurements.

Leaf relative water content (RWC) was measured using the method described by Lafitte (2002). Antioxidant activity of leaves was measured using the 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging method (Prieto *et al.*, 2012) and the Ferric Reducing Antioxidant Potential (FRAP) assay (Benzie and Strain, 1996). Leaf total soluble sugar content was measured according to Dubois *et al.* (1956), and leaf proline content was measured according to Bates *et al.*, (1973) using oven-dried, powdered leaf samples. Chlorophyll a, b, and total carotenoid concentrations were also measured according to Lichtenthaler and Welburn (1983).

C. Data Analysis

The data were statistically analyzed using analysis of variance (ANOVA) for each measurement interval separately, and mean comparisons were carried out using Duncan's Multiple Range Test (DMRT). The drought tolerance index (DTI) for each parameter was calculated by taking relative values for each treatment, using the corresponding values of well-watered plants as the reference (Equation 1), following the method described by Al-Azab *et al.*, (2022).

Example:

$$DTI_p = \frac{X_p}{W_p} \quad \text{Equation 1}$$

Where:

DTI_p = Drought Tolerance Index for Photosynthetic Rate, X_p = Photosynthetic Rate of specific treatment at a particular measurement interval, W_p = Corresponding Photosynthetic Rate of WW condition at the same measurement interval.

The experiment was repeated twice to confirm the trends in the results. The final decision was based on the rank summation index (RSI), a multi-criteria selection method (Abayomi and Abidoye, 2009).

III. RESULTS AND DISCUSSION

The application of SA at 150 and 200 mg L⁻¹ resulted in a significantly higher Drought Tolerance Index (DTI) for photosynthetic rate and stomatal conductance compared to the no-spray treatment in both cultivars under glasshouse conditions. A comparatively higher DTI for transpiration rate was also observed in plants treated with 150 and 200 mg L⁻¹ of SA.

Exogenous application of 150 and 200 mg L⁻¹ of SA significantly improved osmolyte accumulation and antioxidant activity, thereby enhancing the drought tolerance index for immature tea plants. Foliar application of SA at these concentrations was the most effective treatment in significantly mitigating the negative effects of drought on tea plants compared to the no-spray (NS) treatment. Therefore,

considering the environmental impact and cost-effectiveness, the application of SA at the lowest effective concentration, i.e., 150 mg L⁻¹, would be notably effective in reducing the impact of drought on immature tea plants under moderate moisture stress.

The application of 100–200 mg L⁻¹ of ABA significantly improved the Drought Tolerance Index (DTI) for physiological and biochemical parameters, while also enhancing recovery under drought stress conditions. Additionally, ABA caused a significant increase in antioxidant activity under drought stress, thereby protecting the plants from the detrimental effects of drought stress.

Therefore, it can be concluded that the application of ABA at the lowest effective concentration, i.e., 100 mg L⁻¹, can serve as a potential tool to minimize the effects of drought stress in immature tea plants.

Exogenous GA increased plant metabolic activities throughout the drying cycle, resulting in higher productivity while preserving antioxidant capacity, thus reducing the risk of oxidative damage under drought conditions. The concentration of 50 mg L⁻¹ of GA was the most effective for increasing the Drought Tolerance Index (DTI) for gas exchange parameters, antioxidant activity, and recovery after drought in immature tea plants.

According to the cost-benefit analysis of immature tea plants, the highest net profit from the recovery of plants was observed in those treated with 200 mg L⁻¹ of SA, followed by those treated with 150 and 100 mg L⁻¹ of SA.

SA improved both the survival and productivity of immature and mature tea plants under moisture stress conditions. GA enhanced productivity parameters, such as photosynthetic rate and yield components, compared to SA and ABA. The application of ABA positively affected the water relations of tea plants by regulating stomatal behavior. Both SA and ABA increased the antioxidant activity in both mature and immature tea plants.

According to the results of the Rank Summation Index (RSI), the application of SA and ABA provided benefits in terms of improving drought tolerance during the latter part of the drying cycle (14 Days After Spraying (DAS) and 21 DAS) and exhibited positive improvements in recovery after drought.

The cost-benefit analysis of immature tea plants revealed that those treated with 50 mg L⁻¹ of GA and 150 mg L⁻¹ of SA resulted in the highest net profit, achieving 118% and 122% profit compared to the no-spray (NS) treatment. In contrast, the lowest net profit was observed in the 100 mg L⁻¹ ABA treatment at 14 DAS and 21 DAS.

IV. CONCLUSIONS

Exogenous application of SA significantly improved the yield components while enhancing both the survival and productivity of immature and mature tea plants under moisture stress conditions. The exogenous application of ABA stimulated stomatal closure, reduced gas exchange, and improved relative water content (RWC). Additionally, the exogenous application of GA sustained plant metabolic

activities throughout the drying cycle, resulting in higher productivity under moisture stress conditions.

Therefore, considering plant survival, productivity, environmental impact, and cost-effectiveness, the application of SA at 150 mg L⁻¹ is recommended as a potential tool to minimize the effects of drought stress in both immature and mature tea plants.

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INSTITUTION WHERE RESEARCH WAS CARRIED OUT

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BIOACTIVITY OF AN ENDOPHYTIC FUNGUS ISOLATED FROM *Centella asiatica*

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I. INTRODUCTION

Natural products are metabolites and/or by-products produced by microorganisms, plants or animals (Strobel and Daisy, 2003) and they are an unlimited source of novel and potent chemical entities. Secondary metabolites are produced by organisms as a result of adaptation to the environment or as a defense mechanism (Dias et al., 2012). These secondary metabolites are a highly diverse group of natural products and act as an inexhaustible source of leads for many uses such as medicinal and agricultural uses. It is the unique biosynthesis of these natural products, produced by the countless number of terrestrial and marine organisms, that provides the characteristic chemical structures with an array of biological activities.

Several factors such as seasonal availability, and conservation concerns on some rare plant species, have limited the use of plants as sources of natural products (Abdel-Razek et al., 2020). Microorganisms are a valuable source due to their availability, variability, vast diversity, production of unique compounds and various bioactivities (Abdel-Razek et al., 2020). Endophytic fungi are a group of polyphyletic group of microbes that can survive asymptotically in healthy tissues of plants. They colonize internal plant tissues beneath epidermal layers and in intercellular spaces of tissues (Jia et al., 2016). Endophytic fungi provide tolerance to the host plant

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against abiotic and biotic stresses by producing structurally diverse and biologically active secondary metabolites

There is a general need to find new antimicrobials, therapeutic drugs and agrochemicals that are effective, less toxic and have less environmental impact. Here, new natural products and endophytic fungi offer greater opportunities for the development of drug and agrochemical discovery.

The main objective of the current study was to study the in-vitro α -glucosidase inhibitory activity, antifungal activity, antioxidant activity, brine shrimp lethality and phytotoxic activity of solvent extracts and compounds isolated from endophytic fungi associated with *Centella asiatica*.

The study was conducted with the following specific objectives:

- i. To isolate endophytic fungi from the plant leaves.
- ii. To identify the endophytic fungi.
- iii. To screen solvent extracts of endophytic fungi for antifungal, antioxidant, brine shrimp lethality, enzyme inhibitory and phytotoxic activities.
- iv. To isolate compounds from the crude extracts.
- v. To determine antifungal, antioxidant, brine shrimp lethality, enzyme inhibitory and phytotoxic activities of pure compounds and the identification of their structures.

II. METHODOLOGY

Healthy, fresh leaves of *C. asiatica* (L.) Urban (small-leaved creeping form or Wel-gotukola) were collected from a home garden in the Central Province of Sri Lanka. Leaves were triple sterilized with 96% Ethanol (EtOH) for 1 minute, 2.5% Sodium Hypochlorite (NaOCl) for 1 minute, 96% EtOH for 30 seconds, and finally with sterile distilled water. Segments of triple sterilized leaf samples were then placed aseptically on the surface of Petri dishes with potato dextrose agar medium and incubated at room for 5-7 days. Plates were examined for the emergence of fungi from leaf segments and repeated sub-culturing was done to obtain a pure fungal strain of the emerged fungus.

A pure culture of the emerged fungus grown on PDA for about 10-14 days was used for the DNA extraction. DNA extraction was performed using Promega Wizard® Genomic DNA Purification Kit A1120 (Promega Corporation, USA). Internal Transcribed Spacer (ITS) region of the rDNA was amplified using the primer pair ITS 1-F and ITS 4. The amplified PCR products were sequenced using Sanger sequencing and the sequence alignment was done using BioEdit Sequence Alignment Editor (Version 7.2.5). A similarity-based search in the National Centre for Biotechnology Information (NCBI), Basic Local Alignment Search Tool (BLAST) was carried out to find similar sequences.

Large-scale culturing of the fungus was carried out in potato dextrose broth (PDB) media and was allowed for undisturbed incubation for 10 days and then incubated while shaking on a laboratory shaker for 5 weeks until extraction at room temperature. The resulting culture broth was filtered and partitioned with ethyl acetate (EtOAc) (1:1). Mycelium was extracted to EtOAc followed by methanol (MeOH) using ultrasonication to obtain the crude extracts. The combined EtOAc extract of broth and mycelium was chromatographed over silica gel columns, Sephadex LH-20, preparative thin layer chromatography and HPLC to isolate compounds.

The crude extracts and the compounds were tested for the selected bioactivities. Antifungal activity was determined by the TLC bioautography method for *Cladosporium cladosporioides* and *Candida albicans*, antioxidant activity by DPPH radical scavenging activity, antidiabetic activity by α - glucosidase inhibitory activity, cytotoxicity by brine shrimp (*Artemia salina*) lethality assay and phytotoxicity by lettuce seed germination inhibition assay.

III. RESULTS AND DISCUSSION

An endophytic fungus emerged from the cut ends of the leaves of *C. asiatica* placed on PDA plates. The BLAST search of the aligned sequences of the fungus indicated that the sequence matched 98.79% percentage identity with those of *Muyocopron laterale* isolates. Therefore, the endophytic fungus was identified as *Muyocopron laterale*. *Muyocopron laterale* is an Ascomycetes fungi belonging to the order Muyocopronales. *Muyocopron laterale* is not reported to be isolated from *C. asiatica* before. Therefore, this is the first report of *M. laterale* as an endophytic fungus isolated from *C. asiatica*. *Muyocopron laterale* has been isolated as an endophytic fungus in several studies and the bioactivity of some of its metabolites has also been studied. In the current study, only one endophytic fungus was isolated from the leaves of *C. asiatica*, although this plant has shown the ability to harbor a variety of endophytic fungi in previous studies. This could be explained by the fact that population and distribution of endophytes can be affected by several host factors such as genetic background, age, nutrient level and ecological habitats. Further, environmental factors such as location, climate, time of collection, and soil conditions can affect the endophytic population in a plant (Jia et al., 2016).

The broth EtOAc extract (B) showed the highest antioxidant activity while mycelium EtOAc (ME) and mycelium MeOH (MM) extracts showed moderate activity. High α -glucosidase inhibitory activity was shown by ME and MM extracts whereas extract B showed moderate activity. Brine shrimp lethality was shown as moderate by B and MM extracts and ME extract showed weak lethality. Extract B showed the highest root and shoot inhibition while ME extract showed moderate root and shoot inhibition. MM extract did not show any phytotoxicity.

Chromatographic separation of combined EtOAc extract (B and ME) furnished six compounds austdiol (KCCA-3), 4-(hydroxymethyl)-3-methoxy-5-methylcyclopent-2-enone (KCCA-4), (7R,8S)-5-(2-(3,6-dimethyl-4-oxo-4H-pyran-2-yl)propyl)-7,8-dihydroxy-3,7-dimethyl-7,8-dihydro-6H-isochromen-6-one (KCAA-5), eugenitin (KCCA-6), 6-hydroxy-4,6-dimethyl-3-(3-methyl-2,4-dioxohex-5-enyl)-5-oxocyclohex-3-enyl3-hydroxy-2,4dimethylhexanoate (KCAA-7) and 6-methoxymethyleugenin (KCCA-8). KCAA-3, KCAA-6, and KCAA-8 have been previously isolated from endophytic *M. laterale* in different studies (Yuan et al., 2021; Nakashima et al., 2020). KCAA-5 and KCAA-7 have been isolated from endophytic fungi of *Dothideomycete* sp. [7-8]. Therefore, it is evident that these compounds except KCAA-4 are a group of compounds produced by different fungi of class Dothideomycetes, to which *M. laterale* from the present study also belongs to.

Bioactivity studies of the compounds showed that KCAA-7 has antioxidant activity with IC_{50} 64.45 mg L⁻¹, KCAA-6 showed weak brine shrimp lethality (IC_{50} 684.26 mg L⁻¹), KCAA-3 showed root inhibition (IC_{50} 5.38 mg L⁻¹) and shoot inhibition (IC_{50} 805.19 mg L⁻¹). High α - glucosidase inhibitory activity was shown by KCAA-3, KCAA-4, KCAA-5, KCAA-6 (IC_{50} 4.22 mg L⁻¹, 48.43 mg L⁻¹, 22.55 mg L⁻¹ and 55.69 mg L⁻¹ respectively). None of the compounds showed antifungal activity for *C. albicans* while KCAA-8 showed antifungal activity for *C. cladosporioides* up to 125 mg L⁻¹ while showing strong inhibition zones.

IV. CONCLUSION

Some of the tested bioactivities of the crude extracts and compounds have not been previously studied. The presence of α -glucosidase inhibitors from this endophyte leads to the development of anti-diabetic drug discovery. Phytotoxic and antifungal compounds are potential leads for the production of eco-friendly herbicides and antifungal agents respectively. Therefore, the results of this study suggest the potential of this endophytic fungus to produce bioactive secondary metabolites with potential uses in medicine and agriculture. The findings could be used in future research for in vivo studies to evaluate the antioxidant, antidiabetic and cytotoxic potential and field studies to evaluate the phytotoxicity.

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SCIENCE EDUCATION

IMPACT OF TEACHER STUDENT INTERACTION ON SCIENCE LEARNING: A CASE STUDY IN BADULLA EDUCATIONAL ZONE

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I. INTRODUCTION

Education, as a lifelong journey beginning from birth, plays a paramount role in shaping individuals' personal and professional trajectories in today's competitive landscape. It is a continuous process characterized by the acquisition of knowledge, skills, and confidence necessary for navigating life's complexities. Within this educational framework, teachers serve as the linchpin, guiding and nurturing students through their learning endeavors. Particularly in subjects like science, which unlock understanding of the natural world and foster critical thinking, the role of teachers becomes indispensable (Bhardwaj and Vishwavidyalaya, 2016). This research aims to delve into the intricate dynamics of teacher-student interaction within the domain of science education, recognizing its pivotal influence on students' learning experiences and outcomes. By scrutinizing various parameters such as teacher characteristics, student performance, teaching methodologies, and classroom environments, this study seeks to unravel the complexities of science education within the context of the Badulla Education Zone.

The overarching objective of this research is to discern the impact of teacher-student interaction on science learning within the Badulla Education Zone. To achieve this goal, specific objectives have been delineated. Firstly, the research seeks to identify the effect of sub-parameters in teacher-student interactions to enhance science learning outcomes.

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Secondly, it aims to quantify the influence of various parameters and sub-parameters of teacher-student interaction on O/L students' science learning achievements. Lastly, the research endeavors to develop a statistical equation that models optimal teacher-student interactions conducive to improvements in science learning. Through the exploration of these objectives, this research endeavors to shed light on the intricacies of science education practices, offering insights and recommendations for enhancing educational outcomes and fostering student success in the Badulla Education Zone.

II. METHODOLOGY

A. Methodology Overview:

The methodology employed in this study adopts a mixed methods research approach, integrating both qualitative and quantitative methodologies to provide a comprehensive understanding of teacher-student interaction in science education within the Badulla Education Zone. This approach is chosen to mitigate the limitations inherent in singular research methodologies, allowing for a more nuanced exploration of the research problem. By leveraging data from multiple sources, this study aims to achieve depth and breadth in analyzing the dynamics of teacher-student interaction and its impact on science learning outcomes.

B. Research Approach and Design:

Drawing on Creswell's mixed methods research approach, this study employs a convergent parallel design, which involves collecting and analyzing both qualitative and quantitative data separately and then integrating the findings for a holistic understanding (Creswell, 2003). This design facilitates the exploration of teacher-student interaction from various perspectives, enabling researchers to uncover nuanced insights that may not be apparent through a single method alone. Through thematic analysis of qualitative data and statistical analysis of quantitative data, this study endeavors to identify patterns, relationships, and correlations that shed light on the intricacies of science education within the Badulla Education Zone.

The adoption of a mixed methods approach in this study is pivotal as it allows researchers to capture the complexity of teacher-student interaction in science education comprehensively. By combining qualitative insights with quantitative data, researchers can gain a deeper understanding of the factors influencing science learning outcomes, thus informing more effective educational practices. Additionally,

the use of a convergent parallel design enhances the validity and reliability of the findings by triangulating evidence from multiple sources. This ensures that the conclusions drawn are robust and grounded in empirical evidence. Furthermore, the focus on the Badulla Education Zone provides context-specific insights that can inform educational policies and practices tailored to the needs of the local community. Overall, the methodology employed in this study serves as a robust framework for investigating the intricacies of science education and advancing knowledge in the field.

III. RESULTS AND DISCUSSION

The study delved into the intricate relationship between various factors affecting science learning outcomes, particularly emphasizing teacher characteristics, teaching-learning processes, and classroom environment. Both qualitative and quantitative analyses provided rich insights, shedding light on key determinants of students' science scores.

A. Quantitative Analysis Findings:

The quantitative analysis unearthed a significant positive correlation between teacher characteristics, teaching-learning processes, and students' science scores. Notably, parameters such as punctuality, management skills, friendliness, subject knowledge, and communication skills were identified as pivotal. Furthermore, the study revealed a strong association between students' performance indicators like parental support, healthiness, active participation, and interest in the subject with their science scores. These quantitative findings underscored the multifaceted nature of factors influencing science learning outcomes, emphasizing the need for a comprehensive approach.

B. Regression Analysis

The study employed multiple regression analysis to predict science scores based on teacher characteristics (X_1), student performance (X_2), teaching-learning processes (X_3), and classroom environment (X_4). The regression equation derived from the analysis is as follows:

$$Y = -15.65 + 6.489X_1 + 15.27X_2 + 6.037X_3 + 1.573X_4 \quad (1)$$

This equation indicates the change in science scores for every one-unit change in the respective predictor variables. The coefficients provide insights into the magnitude of impact each variable has on science scores.

Overall, the study's findings provide valuable insights into the multifaceted nature of factors influencing science learning outcomes and offer actionable recommendations for improving science education practices and policies.

C. Qualitative Analysis Insights

The qualitative analysis complemented the quantitative findings by providing nuanced perspectives on teacher-student interactions and classroom dynamics. Themes such as parental support, healthiness, active participation, interest in

the subject, and practical work emerged as crucial influencers of science scores. Both teachers and students highlighted the significance of these factors, emphasizing their impact on student engagement and understanding.

D. Convergence of Qualitative and Quantitative Findings:

The convergent analysis revealed a harmonious relationship between qualitative and quantitative results, consolidating the understanding of factors influencing science learning outcomes. While qualitative insights provided depth and context, quantitative data offered statistical validation, enhancing the robustness of the findings. The convergence highlighted the pivotal role of teacher-student interactions, teaching strategies, and classroom environment in fostering conducive learning environments for science education. Overall, the convergence of qualitative and quantitative findings illuminates the multifaceted nature of factors influencing science learning outcomes.

These results have significant implications for educational practice and policy. They highlight the imperative for targeted interventions aimed at enhancing teacher-student interactions, promoting active student engagement, and optimizing classroom environments to foster academic success in science education. Furthermore, the findings underscore the need for continuous professional development programs to equip educators with the necessary skills and knowledge to create enriching learning experiences. By addressing both teacher characteristics and student performance indicators, educational stakeholders can work collaboratively to cultivate a supportive ecosystem that nurtures students' curiosity, critical thinking, and passion for science. Ultimately, these efforts are essential for cultivating a generation of scientifically literate individuals capable of addressing complex global challenges and driving innovation in the 21st century.

IV. CONCLUSIONS

The study conducted in the Badulla Education Zone delved into the impact of teacher-student relationships on science learning, employing a mixed methods approach and convergent research design for robust analysis. Through various analyses, it was observed that teacher characteristics, such as punctuality, management skills, subject knowledge, and communication skills, significantly correlate with students' science scores. Additionally, parameters affecting the teaching-learning process, including teaching methods, strategies, and feedback mechanisms, play a pivotal role in enhancing science learning outcomes.

The findings underscored the importance of fostering positive teacher-student interactions, wherein students feel valued, respected, and motivated. Positive reinforcement, coupled with effective teaching methods and aids, creates an engaging learning environment conducive to optimal student performance. Moreover, formative and summative assessments, when employed effectively, not only gauge student progress but also contribute to continuous improvement in the teaching-learning process. Furthermore, the study revealed that students' performance is strongly linked to their science scores, emphasizing the significance of

nurturing students' interest and active participation. While parental support and student health exhibit weaker associations with science scores, practical classes were found to enhance students' understanding and interest in scientific concepts.

Regarding the impact of parameters on teacher-student interaction, regression analysis illustrated the varying degrees of influence. Teachers' performance emerged as a significant predictor of science scores, followed by students' performance, while the classroom environment showed a comparatively lesser effect. In conclusion, the study advocates for the cultivation of a learning environment characterized by strong teacher-student relationships, recognizing the individual needs and aspirations of students. By prioritizing effective communication, supportive interactions, and engaging teaching practices, educators can foster a culture of learning that nurtures students' academic success and personal growth in the realm of science education.

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STATISTICS AND COMPUTER SCIENCE

MIXED POISSON DISTRIBUTIONS AND POISSON REGRESSION MODELS FOR OVER-DISPersed COUNT DATA

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I. INTRODUCTION

In scientific research, the importance of modeling the count data has increased during the last decade. Some major fields are actuarial science, biomedical science, economics, engineering, and social science. In this context, the Poisson distribution and its regression model are the standard tools to analyze the univariate count data and count responses with covariate-dependent observations, respectively (Johnson et al., 2005). The probability mass function (PMF) of the Poisson distribution with parameter μ is given as;

$$f_{Y|M}(y|\mu) = \frac{e^{-\mu} \mu^y}{y!}; y = 0,1,2,\dots; \mu > 0 \quad (1)$$

Even though the Poisson distribution is very flexible to model the count data, in some real-world applications, the Poisson distribution fails to match with empirical observations due to the variance of the observed data exceeding the theoretical Poisson variance. This phenomenon is explained as over-dispersion. Further, since the Poisson regression model is a generalized linear model (GLM), its maximum likelihood estimator (MLE) shows instability when the covariates are highly correlated. It is commonly referred to as a multicollinearity issue (Frisch, 1934).

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The primary objectives of the study were to develop a mixed Poisson (MP) distribution for univariate over-dispersed count data and develop its regression models for two types of count data;

- i. Over-dispersed count responses with a set of linear-independent covariates.
- ii. Over-dispersed count responses with a set of highly correlated covariates.

To achieve the primary objectives, the following specific objectives were considered;

- i. To explore a computationally flexible and efficacious continuous distribution for modeling the Poisson parameter.
- ii. To develop a new MP distribution by mixing the Poisson distribution and the explored continuous distribution in (i), and its regression model.
- iii. To develop the MLEs for the estimation of unknown parameters of the developed MP distribution and its regression model in (ii).
- iv. To examine the asymptotic accuracy of the MLEs developed in (iii) by designing Monte Carlo simulation studies.
- v. To compare the performance of the developed MP distribution and its regression model in (ii) with the existing predecessors with respect to distributions' flexible structural properties and applications of simulated and real-world data sets.
- vi. To develop possible biased estimators for the developed MP regression model in (ii) based on sample information.
- vii. To do theoretical comparisons among the developed biased estimators in (vi) with respect to the mean square error (MSE) matrix criterion.
- viii. To compare the performance of the proposed biased estimators in (vi) by designing Monte Carlo simulation studies and using applications of simulated and real-world data sets.

II. METHODOLOGY

Due to the fact that the resultant MP distribution has an explicit form of the PMF and works efficiently when we model the Poisson parameter by a Lindley family of distributions, we explored a Lindley family of distributions that can perform well for various characteristics of data sets by conducting a comprehensive comparative study. From the comparison study, we could explore an efficacious Lindley

family of distributions and named as Modified Quasi Lindley (MQL) distribution. It is a two-component mixture of exponential and gamma distributions and its density function was derived as;

$$f_M^{MQL}(\mu) = \frac{\theta e^{-\theta\mu}}{(\alpha^3+1)\Gamma(\gamma)} (\Gamma(\gamma)\alpha^3 + (\theta\mu)^{\gamma-1}) \quad (2)$$

$$\mu > 0, \theta > 0, \alpha^3 > -1, \gamma > 0.$$

Then, we developed the PMF of the new MP distribution by amalgamating the Poisson and MQL distributions as follows;

$$f_Y^{MQL}(y) = \int_0^\infty f_{y|M}(\gamma|\mu) f_M^{MQL}(\mu) d\mu \quad (3)$$

$$= \frac{\theta(\Gamma(\gamma)\Gamma(\gamma+1)\alpha^3(1+\theta)^{\gamma-1} + \theta^{\gamma-1}\Gamma(\gamma+\gamma))}{y!(\alpha^3+1)(1+\theta)^{y+\gamma}\Gamma(\gamma)}$$

$$y = 0, 1, 2, \dots, \theta > 0, \gamma > 0, \alpha^3 > -1.$$

This new MP distribution was named as Poisson modified Quasi Lindley (PMQL) distribution. Further, we developed some important structural properties of PMQL distribution and regression model based on the GLM approach.

Finally, in order to mitigate the multicollinearity issue in the PMQL regression model, we proposed some notable biased estimators based on sample information. They are PMQL ridge, Liu, almost unbiased ridge, almost unbiased Liu, and optimal generalized regression estimators. Here, we discussed the asymptotic properties and theoretical comparisons based on the MSE matrix criterion of these estimators.

III. RESULTS AND DISCUSSION

From the PMF of the PMQL distribution, we can show that it can accommodate the negative binomial and geometric distributions. Its shape properties revealed that distribution is capable of covering the unimodal and bimodal cases at its various parameter settings. We could prove that its hazard rate function accommodates the bathtub and monotonic increasing and decreasing shapes. Its variance function indicates that the variance of the distribution is greater than its mean. Then, it can explain the over-dispersion. Further, properties of the Skewness, kurtosis, and index of dispersion functions revealed that it can accommodate various ranges of horizontal symmetries, right-tail heaviness, and heterogeneity for over-dispersed count data. By using Monte Carlo simulation studies, we could show that the MLEs for the PMQL distribution and regression model satisfy the asymptotic property.

We used four real-world and three simulated over-dispersed datasets in order to show the goodness of fits of PMQL distribution and regression model. From the goodness of fit test results, we could show that the distribution and its regression model perform better than other notable existing MP distributions and their regression models.

From the result of Monte Carlo simulation study and a real-world application, it was concluded that;

- i. The biased estimators show a better performance than the MLE when multicollinearity exist.

- ii. The performance of biased estimators are affected by the factors of degrees of correlation among the covariates, the sample size, the value of the intercept, the number of covariates, and values of the over-dispersion parameters of the PMQL regression model.
- iii. The optimal generalized regression estimator performs well compared to other given biased estimators.
- iv. Results obtained from the simulated and real-world applications are consistent with the theoretical and simulation study results.

IV. CONCLUSIONS

The center of interest of this study was to propose an alternative and computationally flexible MP distribution for univariate over-dispersed count data and its regression models based on several regression coefficient estimators for two types of count data sets as indicated in the introduction section.

The fundamental structural properties of the proposed MP distributions and its regression model have been derived and studied. Further, the superiority conditions for the proposed regression coefficient estimators were also derived by means of the MSE matrix criterion. Moreover, the consistency of theoretical findings has been examined with Monte Carlo simulation studies and simulated and real-world applications. We hope the results of this study will be more helpful for the further development of the MP distributions and their regression models.

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INSTITUTION WHERE RESEARCH WAS CARRIED OUT

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CLASSIFICATION AND ANALYSIS OF ALZHEIMER'S DISEASE USING DEEP LEARNING ON NEUROIMAGING DATA

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I. INTRODUCTION

Alzheimer's disease (AD) is an irreversible, progressive, neurodegenerative illness that affects primarily older individuals. AD is characterized by a higher impairment in memory or cognitive skills than healthy adults of the same age. AD is, therefore, a major public health concern in many parts of the world, including Sri Lanka. Early detection of Alzheimer's disease is important, especially at pre-symptomatic stages, to slow down or prevent disease progression. Clinical, neuropsychological, and neuroimaging assessments are the current diagnosis methods for AD, but a reliable *in vivo* diagnosis is not in sight yet. Since the invention of the brain connectome, many studies have utilized structural and functional connectomes and advanced graph-theoretical approaches to investigate Alzheimer's brain networks and network-based measures. Deep neural networks have produced state-of-the-art outcomes in a variety of medical imaging applications, including the identification of Alzheimer's disease using neuroimaging data.

The aim of this work is to elucidate the changes in structural connectivity within the brain, including the asymmetric patterns between the left and right hemispheres,

in Alzheimer's disease. This improved understanding holds promise for the development of novel biomarkers and more effective treatment strategies for AD patients.

To achieve the aims, a study was conducted with the following specific objectives:

- i. Generating structural brain networks using diffusion MRI and T1 images for AD patients and healthy normal controls (NC).
- ii. An efficient Convolutional Neural Network (CNN) framework is developed to accurately detect AD, analyze CNN's classification choices, and identify discriminative changes in white matter connectivity.
- iii. An efficient CNN-based approach investigates asymmetrical white matter changes in AD using left and right hemispherical brain networks.
- iv. Graph Neural Network (GNN) architecture is employed for AD detection, leveraging the natural graph representation of structural brain networks.

II. METHODOLOGY

A. Structural Connectomes

The diffusion-weighted MR and T1-weighted MR brain images of 100 AD and 100 NC were selected from the Alzheimer's Disease Neuroimaging Initiative (ADNI) project database (<http://adni.loni.usc.edu/>). Fig. 1 illustrates the pipeline of a structural brain network construction, which includes preprocessing, brain parcellation, and whole brain fiber tractography. Eighty cortical and sub-cortical regions were created, with each region representing a node and the tractography stream lines connecting different cortical and subcortical regions were considered as edges of the brain network. Adjacency matrices of weighted graphs with 80×80 elements were created for each subject. Then the left and right hemispherical networks (40×40) were created using the whole-brain networks for the analysis of brain asymmetry patterns in AD.

B. CNN Architecture

The proposed CNN architecture is depicted in Fig. 2. It contains three convolutional layers and three fully-connected layers. A dropout layer and an average pooling layer are located in between fully-connected and convolutional layers, respectively. In fully connected and convolutional layers, the Rectified Linear Activation Function (ReLU) was used to prevent the exponential growth in the computation. In the last fully-connected layer, we employed the softmax activation function to obtain the classification scores. The input of the architecture is the structural brain network and output is the classification scores of AD and NC. Based on the validation results, we have tuned the values of hyper-parameters.

C. Visualizing the CNN Classifier's Decision using Grad-CAM

Based on the Gradient-weighted Class Activation Mapping (Grad-CAM) technique, we have developed an approach to determine the most influential structural white matter brain connectivity in classifying AD patients from NC subjects. The high-resolution class activation map is used to identify the feature map regions that are influenced to detect AD in classification. Finally, an average of these heatmaps and high-resolution class activation maps were generated.

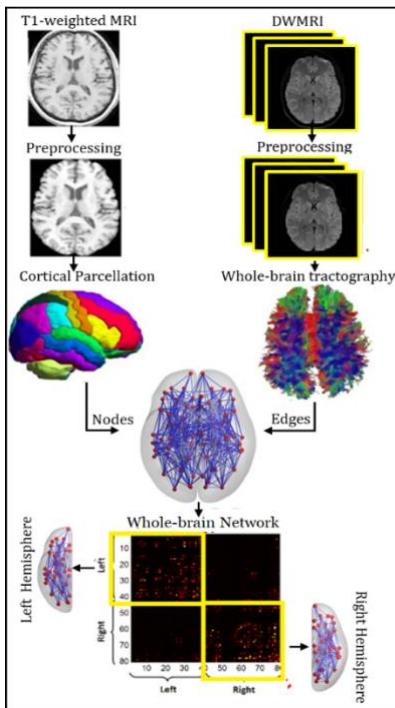


Fig. 1: The major steps of creating a structural brain network

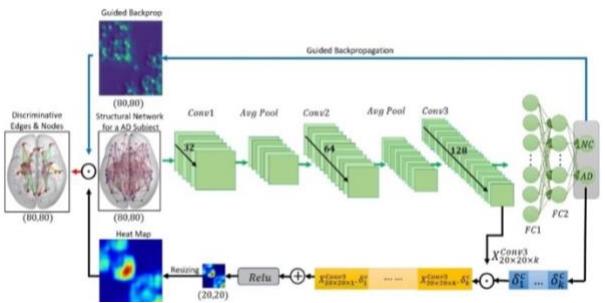


Fig. 2: Proposed CNN Architecture and CAM visualization for left

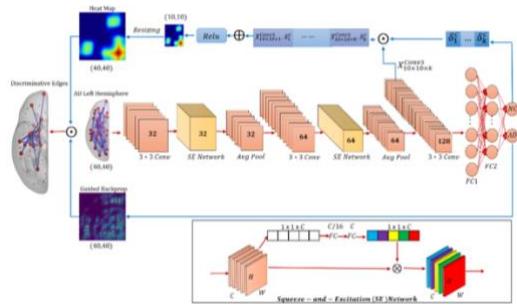


Fig. 3: Proposed CNN Architecture and CAM visualization for AD and NC

D. CNN Architecture for Hemispheres

The proposed CNN model consumes the structural brain network of the left or right hemisphere with dimensions of 40×40 and outputs the classification scores for the AD and NC classes. As illustrated in Fig. 3, a squeeze-and-excitation (SE) network block and an average pooling layer are employed between each convolutional layer to enrich AD-specific features and reduce feature dimensionality.

E. Discriminative Asymmetrical Changes

A visualization technique based on the Grad-CAM approach was developed to generate activation maps for the AD class on the input data. Then, the corresponding regions in the structural brain network and white matter pathways were identified. These processes were conducted separately for the left and right hemispheres using their corresponding CNN models.

F. GNN Architecture

In contrast to previous work, we introduce a GNN-based AD classification model that effectively captures the features of the structural brain networks. The proposed GNN contains four different types of layers, as seen in Fig. 4 graph convolutional layers, sort pooling layers, one-dimensional convolutional layers, and fully-connected layers. It consumes the structural brain graph as the input and produce the classification scores of AD and NC.

III. RESULTS AND DISCUSSION

We have successfully designed the CNN model for the structural brain network and showed the experimental results with 95% accuracy. Fig. 5 shows the result of the class activation map and discriminative nodes and edges, which illustrates discriminative connectivity in regions. A distinct pattern was discovered, revealing significant white matter changes within the temporal/subcortical regions and between the temporal/subcortical regions and the frontal and parietal regions.

By applying the proposed CNN model for hemispheres, an average classification accuracy of 97% for the left hemisphere and 95% for the right hemisphere was achieved. As illustrated

in Fig. 6, we have identified discriminative asymmetrical changes, including distinct connectivity changes within the left and right hemispheres. The proposed GNN architecture with structural brain graphs classified AD at a high accuracy of 97%.

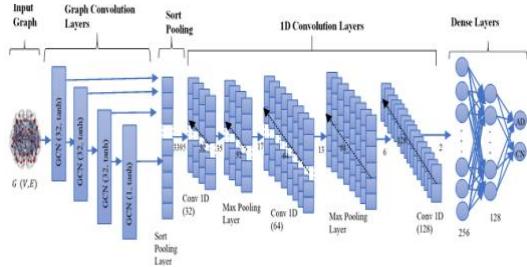


Fig. 5: Proposed GNN Architecture

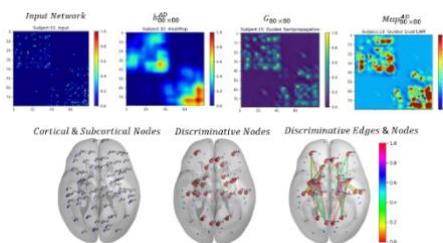


Fig. 7: Visualization of an AD subject

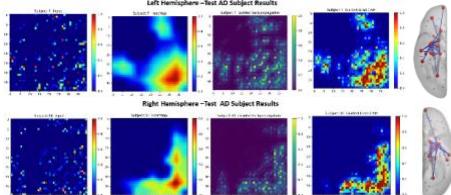


Fig. 7: Visualization of an AD subject of the left and right hemispheres

The nature of AD is neurodegeneration, which results in brain network dysfunction.

IV. CONCLUSIONS

The CNN and GNN models were successfully developed to classify AD from NC using the structural brain network. This research visually explains the Grad-CAM based CNN decision in structural brain network classification to show discriminative white matter connectivity variations in AD and distinct connectivity changes in the left and right hemispheres. Our research contributes to improving diagnostic accuracy and enhancing our understanding of the structural changes in AD and asymmetric pattern in hemispheres.

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RECENT CHANGING CHARACTERISTICS OF WET AND DRY PATTERNS IN BADULLA, SRI LANKA

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I. INTRODUCTION

In recent decades, the global climate system has undergone unprecedented changes due to global warming. It is essential to understand and predict climate change, as it affects decisions related to the economy, food security, water resource management, policy planning, and disaster preparedness of the country.

The wet and dry patterns can be useful indicators in representing the variability of daily precipitation and the weather patterns of the country. The characteristics of wet and dry spells have been analyzed globally, focusing on two major aspects. The first aspect relates to the spatial distribution and temporal trends of wet and dry spells, while the second aspect focuses on curve fitting of probability distributions for wet and dry spells.

The main objective of the present work was to identify recent changes in the wet and dry weather patterns based on daily precipitation from 1992 to 2021 in the Badulla region, Sri Lanka. This was achieved by analyzing the trends of daily, annual, and seasonal rainfall, wet and dry state transition probabilities, and wet and dry spells in the region.

The study was conducted with the following specific objectives:

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- i. Investigate the recent trends in daily, annual, and seasonal rainfall in the region
- ii. Identify the variations in dry and wet state transition probabilities over the period
- iii. Investigate the recent changes in wet and dry spells of the region
- iv. Find the best-fitting distribution to describe the occurrence of daily precipitation events in the region
- v. Propose the Gaussian Mixture Distribution (GMD) as an alternative model to describe the occurrence of daily precipitation events in the region

II. METHODOLOGY

The region being studied is Badulla, located in the Uva Province of Sri Lanka, with geographical coordinates of $6^{\circ} 59' 2.99''$ North latitude and $81^{\circ} 03' 13.80''$ East longitude.

The data used in this study was collected from the Department of Meteorology, Colombo. To identify recent changes, the entire data series (1992–2021) was divided into three consecutive decades: 1992–2001, 2002–2011, and 2012–2021.

The methodology for analyzing wet and dry patterns in the Badulla region consists of five major steps.

Step 1: Trend analysis using the Mann-Kendall (MK) test statistic and Sen's Slope Estimator

- Trends in daily, annual, and seasonal rainfall
- Trends in annual wet and dry days

Step 2: Identify the variations in dry and wet state transition probabilities over the period

- Monthly variation
- Seasonal variation
- Variation across consecutive time periods

Step 3: Wet and dry spell analysis

- Analysis of the sequence of wet and dry spells
- Analysis of mean wet and dry spell durations

Step 4: Find the best-fitting distribution to describe the occurrence of daily precipitation events in the region

Step 5: Propose GMDs as an alternative model to describe the occurrence of daily precipitation events in the region.

III. RESULTS AND DISCUSSION

The results of the trend analysis showed an increasing trend in daily precipitation from 1992 to 2021. However, the rainfall series within the three separate consecutive decades did not show any increasing or decreasing trends and remained constant over each decade. When looking at the seasonal variations in precipitation, only the southwest monsoon season exhibited an increasing trend, with a slope of 9.0714 mm/year. In contrast, the other three seasons did not show any increasing or decreasing trends within the specified timeframe. Furthermore, the annual wet days also showed an increasing trend, with a slope of 0.86 days per year, while there was a decreasing trend in annual dry days from 1992 to 2021.

Following this, wet and dry state transition probabilities were calculated using the observed monthly, seasonal, and decadal wet and dry series of the region. According to the observed transition probabilities, $P(W/W)$ and $P(D/D)$ showed a bimodal pattern of rainfall occurrence with two distinct peaks. Furthermore, the observed wet and dry state transition probabilities for all four monsoonal seasons increased significantly over the consecutive decades. When comparing the three consecutive decades, the highest state transition probabilities were observed in the most recent decade compared to the past two decades in all four seasons. The results indicated that the probabilities of having two consecutive dry or wet days increased significantly over time. Thus, the recent decade experienced the highest number of consecutive dry or wet days compared to the past two decades. The results of the Chi-square test statistic also indicated that the observed transition probability matrices, calculated separately for the three consecutive decades, were significantly different at the 5% significance level.

To gain a better understanding of periods with more than two consecutive wet or dry days, wet and dry spells were tabulated and analyzed. Overall, the number of dry spells in the region was relatively higher than wet spells across all three decades. This indicates that the region experienced more consecutive dry days than consecutive wet days. When looking at longer dry spells, the highest number occurred in the recent decade, with 7 instances where dry spells exceeded 30 days, compared to 4 instances in each of the past two decades. Additionally, the recent decade witnessed the highest mean dry and wet spell durations (5.31 and 3.31 days, respectively) compared to the previous two decades. Similar trends were observed across all four monsoonal periods of the year. In conclusion, the wet and dry spell analysis indicates that, during the last decade, daily precipitation in Badulla experienced longer durations of both wet and dry spells compared to the past two decades.

To identify the inherent variability in the wet and dry patterns of the region, the best-fitting probability distributions were determined. First, single theoretical distributions, namely NBD, LND, GD, and ED, were fitted. Prior to this, the distributions of wet and dry spells in the region for the three decades were compared using the Kolmogorov-Smirnov test. The results indicated that both wet and dry spells followed different distributions in all three consecutive decades at the 5% significance level. According to the calculated goodness-of-fit statistics (AIC, BIC, RMSE, and MAPE), the Log-Normal distribution provided the best fit for both wet and dry spells in the region across all three decades.

In the probability distribution literature, a single distribution sometimes does not provide a proper fit; however, a mixture of distributions can yield better results. Thus, Gaussian Mixture Distributions (GMDs) were proposed and fitted to the sequences of dry and wet spells in the region. The GMDs demonstrated better goodness-of-fit test results than single distributions for nearly all dry and wet spell sequences in the region. Similarly, the GMDs were the most frequently selected theoretical distribution to describe the characteristics of wet and dry spells in all four seasons of the year. However, among the fitted models, the Log-Normal Distribution (LND), which had the least number of estimated parameters, was also a successful probability model for representing the sequence of wet and dry days in daily rainfall occurrences.

To identify the recent changes in the distributions of wet and dry spells, the distributions within each decade were analyzed separately. The Kolmogorov-Smirnov test statistics demonstrated that the three decades exhibited completely different distributions for wet and dry spells. The fitted distributions also indicated that there were distinct distributions in wet and dry spells over the three recent decades. Finally, it can be concluded that the distributions of wet and dry spells changed over time.

IV. CONCLUSIONS

There has been a significant increase in daily precipitation over the past three decades. Furthermore, there has been a notable increase in state transition probabilities over this period. The highest transition probabilities were observed in the recent decade. Thus, the probabilities of having two consecutive dry or wet days have increased significantly. The region has experienced longer wet and dry spells compared to the past two decades. As a result of this trend, there could be an increased risk of extreme weather conditions, such as floods and droughts, in the region in the future. Gaussian Mixture Distributions were the most suitable distributions to describe the sequence of wet and dry spells in all four seasons of the year. However, in terms of the number of estimated parameters, the Log-Normal Distribution was also a successful probability model. When comparing the three decades separately, completely different distributions were observed in wet and dry spells over the last three decades. Overall, the study indicates that the sequence of wet and dry days in daily rainfall occurrences has significantly changed in the Badulla region over the last three decades.

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Postgraduate Institute of Science, University of Peradeniya, Sri Lanka.

ZOOLOGICAL SCIENCES

HATCHING SUCCESS, NEST TEMPERATURE AND EMBRYONIC DEVELOPMENT OF HATCHERY RELOCATED EGGS OF GREEN (*Chelonia mydas*) AND OLIVE RIDLEY (*Lepidochelys olivacea*) TURTLES

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I. INTRODUCTION

Sea turtles have played crucial roles in maintaining healthy oceans worldwide for millions of years. Sea turtles live in marine or estuary areas and are members of the Order Testudines, Suborder Cryptodira. Five of the world's seven sea turtle species nest in Sri Lankan beaches, mainly on south and southwestern coasts. The Green turtle, Leatherback, Olive Ridley, Hawksbill, and Loggerhead Turtle are among them.

Sea turtle populations have experienced significant declines in recent centuries, with many species becoming ecologically extinct before becoming physically extinct. Sea turtles face numerous threats worldwide, including direct and indirect harvesting of adults, juveniles, and eggs, light pollution, climate change, and habitat loss. Nesting habitats are also threatened by mechanical sand pumping, sand mining, oil and gas exploration, and by-catch in fisheries. Nesting habitats are altered by mechanical sand pumping, sand mining, and the consumption of plastic bags, pellets, lines, ropes, latex balloons, aluminium, paper, cardboard, Styrofoam, and rubber. By-catch in fisheries is a key cause of mortality and population decreases in numerous sea turtle species worldwide. Predators target sea turtles at all stages of development, from egg to fully developed stage.

All seven species of sea turtles in the world today are included in the IUCN Red List of Threatened Animals.

Legislative protection of these animals is implemented at both international and national levels. Sea turtle conservation can be accomplished in two ways: in-situ and ex-situ. Ex-situ conservation refers to preserving animals outside their native habitat, whereas in-situ conservation refers to maintaining animals within their natural habitat. Regarding turtle conservation, leaving the eggs in place and reducing the impact of predators and poachers is arguably the best conservation activity on the nesting beach (Miller, 1997).

Relocating eggs to safeguard them from flooding, predation, and poaching is more successful in some cases than leaving them in the original nest. Ex-situ conservation involves relocating eggs outside of their usual nesting locations. Turtle eggs are reburied and protected in turtle hatcheries until the hatchlings emerge. In Sri Lanka, many hatcheries are located along the southwestern coast, where the major nesting of sea turtles occurs. Eggs are relocated in hatcheries as an ex-situ conservation tool for sea turtles.

Nest success and the sex ratio of the hatchlings of sea turtle nests are critical determinants of successful conservation management. The temperature of a sea turtle nest during incubation is vital to the successful development of hatchlings and the determination of their sex. All the current hatcheries are privately-owned tourist attractions, and their contribution towards the conservation of sea turtles is highly debated.

OBJECTIVES

The main objective of the study was to determine the nest success of the hatchery relocated eggs of the two most common sea turtles: Green turtles and Olive ridley turtles nesting on the southwestern coast of Sri Lanka. The specific objectives are:

- To determine the factors affecting the hatching success and incubation duration
- To assess the nest temperature and sex ratio
- To examine the embryonic development of hatchery-incubated sea turtle eggs of Green and Olive ridley turtles

II. METHODOLOGY

Field data collection was carried out in a hatchery named “Sea turtle project Bentota” on the southwestern coast of Bentota South. After explaining the objectives of the study, verbal consent was obtained from the hatchery owner to collect data from egg collectors and hatchery keeper including time gap between collection and reburial of eggs the in-hatchery premises, clutch size, transportation mode, transport distance from the site of excavation to hatchery and the container type used to transport the eggs.

Nests were excavated five days after the first hatching emergence. The dead hatchlings, egg shells and un-hatched eggs were taken out from the nest. The number of dead hatchlings and un-hatched eggs were taken out from the nest, counted and preserved in 10% formalin for further studies. The hatching success was calculated and incubation duration was recorded.

Inside the hatchery, two incubation pens were selected: one under 70% tree shade (shady) conditions and the other under direct sunlight (sunny) in the same hatchery enclosure. Clutches selected for the nest temperature study were equally divided and reburied in the two pens while maintaining the nest depth 65 ± 5 cm for Green turtles and 45 ± 5 cm for Olive ridley turtles. Data loggers were programmed to record the temperature every 60 min at 0.5°C increments, wrapped in a plastic bag and placed in the middle of the nest at the time of the reburial of eggs. In the following phase, eight Green turtle clutches were equally divided and reburied in the two pens; data loggers were placed in the bottom (10 cm from the base), middle (35 cm from the base) and the top (55 cm from the base) of each nest.

Preserved eggs were dissected in the laboratory and initially observed with the naked eye to check the presence of embryonic development. If there's no vicinity of embryonic development to the naked eye, egg yolk was carefully observed under the dissecting microscope to check for early stages of development. The presence of malformations in embryos and dead hatchlings was recorded. The stages of development were determined according to Miller's embryonic staging criteria for sea turtles (Miller et al., 2017). Identifying and categorizing malformations were done according to previously available literature (Makris et al., 2009; Bárcenas-Ibarra et al., 2015).

III. RESULTS AND DISCUSSION

Data was gathered from 100 Green turtle nests in the hatchery with a total of 5,524 eggs and 29 Olive ridley nests with 1,860 eggs. The mean hatching success of Green turtles and the Olive ridley turtles eggs was 89.9% and 84.2%, respectively. According to the probit regression, hatching success of Green turtle eggs was not significant to the given factors studied at the cut-off value of 90%. Correspondingly, Olive ridley eggs also didn't show any significance in hatching success to the given factors with the cutoff value of 84.2%. The incubation duration of Green turtle eggs with a cutoff value of 50.6 days and the incubation duration of Olive ridley with a cut off value of 48.9 days didn't show any

significance to the given factors. According to chi-squared test, Eggs that were transported using buses were more likely to have low hatching success than those were transported via bicycles and by walking (85.4% to 90.2% and 93.4%). Similarly, for Olive ridley that were transported using buses were more likely to have low hatching success than those were transported via bicycles and by walk (82.3% to 82.5% and 87.9%). Collecting eggs from nearby beaches and reburying them in hatcheries as soon as they were excavated from the wild should be followed to avoid long distance travels via unhealthy transport methods.

There was no significant difference between the percentage hatching success of incubated in the sunny ($M = 90.0\pm5.7$) and shady ($M = 89.5\pm6.5$) incubation pens ($B = -0.002$, $p = 0.935$). There was no significant difference between the hatching success of Olive ridley eggs incubated in the sunny ($M = 87.0\pm4.8$) and shady ($M = 86.6\pm6.5$) incubation pens ($B = -0.05$, $p = 0.874$). Mean incubation duration (days) of the Green turtle clutches incubated in sunny incubation pen ($M = 47.8\pm1.6$) was significantly shorter ($B = -1.638$, $p = 0.002$) than that of the shady incubation pen ($M = 52.9\pm1.9$). Mean incubation duration of the Olive ridley clutches incubated in sunny incubation pen ($M = 48.5$, $SD = 1.9$) was significantly shorter ($B = 1.72$, $p = 0.003$) than the shady incubation pen ($M = 51.6$, $SD = 1.7$). Despite the significant temperature difference between the two incubation pens resulting clutches incubated in the shaded pen were on average $\sim 3.3^{\circ}\text{C}$ cooler than the unshaded, there was no significant difference between the hatching successes.

Our results suggest a possibility of 100% female-biased hatchling populations based on TSP temperature for clutches incubated in the sunny incubation pen for both Green and Olive ridley turtles. Whereas, of the 14 clutches incubated in the 70% shaded incubation pen possibly resulting with 14.3% of M: F = 59:41, 28.6% of M: F = 35:65, 42.8% of M: F = 5:95 and only 14.3% of 100% females for Green turtle. Of the 13 Olive ridley clutches incubated in shady incubation pen 7.7% assumed to have a progeny of M: F = 65:35, 7.7% to be M: F = 59:41, 15.4 % to be M: F = 35:65, 30.8% to be M: F = 5:95, 30.8% to be M: F = 5:95, 23.0 % to be M: F = 0.05:99.5 and 15.4% to be 100% female-biased. The mean vertical thermal gradient along the clutches incubated in the shady incubation pen ranged 33°C to 29.5°C from top to bottom with a $2.5\text{-}3.0^{\circ}\text{C}$ difference, while clutches incubated in the sunny incubation pen ranged 36°C to 30°C obtaining much higher thermal variation of $4.5\text{-}5.5^{\circ}\text{C}$ within the nests. These results highlight the importance of shading to reduce the nest temperatures in hatchery relocated sea turtle eggs to a pivotal range to expect 1:1 hatchling sex ratio.

Congenital malformations were observed in embryos at stages 26 - 30 and dead hatchlings. Nineteen malformed Green turtle embryos, which account 5.9% of the total unhatched/dead eggs and 0.64% of the total number of eggs, were detected. There were five malformed Olive ridley embryos, which is 3.8% of the total unhatched/dead eggs and 0.5% of the total number of eggs. Malformations types observed in Green turtle embryos and dead hatchlings were

leucism (4.2%), bicephaly (4.2%), anophthalmia (20.8%), compressed carapace (4.16%), misshapen scutes (45.83%), supernumerary scutes (16.66%) and misshapen bones (4.16%). Malformations types observed in Olive ridley turtle embryos and dead hatchlings were leucism (22.2%), exencephaly (11.11%), laterognathia (11.11%),

development and malformations of sea turtles in Sri Lanka and the first record of schistosomusreflexus syndrome presence in sea turtles of Asia.

IV. CONCLUSIONS

This study highlights the importance of reburying the eggs in nearby hatcheries as soon as they were excavated from the wild to avoid long distance travels via disturbing transport methods and the need to replace polythene bags to firm containers to carry eggs from the excavation site, which can result movement-induced mortality.

Despite the ability to result in high hatching successes even under the increased temperatures, it is important to reduce nest temperatures to a pivotal range to the expected 1:1 hatching sex ratio. Shading can be applied strategically to optimize hatching production with favourable sex ratios to achieve both short-term conservation goals and long-term population sustainability. Further studies need to be carried out in natural nests on the same beach to compare any differences with the hatchery conditions on malformations.

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anophthalmia (11.11%), schistosomusreflexus syndrome (22.2%), misshapen scutes (33.33%).

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INSTITUTION WHERE RESEARCH WAS CARRIED OUT

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ECOLOGY OF SAMBAR DEER (*Rusa unicolor unicolor*) IN HORTON PLAINS NATIONAL PARK, SRI LANKA

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I. INTRODUCTION

Concerns about the ecological impact of Sambar deer in Horton Plains National Park (HPNP), Sri Lanka, arise from their grazing behavior, which affects the park's grasslands and montane cloud forests. These deer, traditionally browsers, have been observed grazing and causing bark damage, thereby threatening forest health. The increasing Sambar population may be linked to the palatability of introduced grass species. Studies reveal their significant ecological impact, including habitat degradation and tree mortality due to bark damage, which can lead to stem defects.

To address these concerns, a comprehensive study was conducted from 2018 to 2022, focusing on the population size, structure, and ecological impacts of Sambar in HPNP. This research aims to provide insights for effective Sambar management and habitat conservation within the park. Understanding their population dynamics and ecological effects is crucial for developing management strategies and ensuring the long-term conservation of Horton Plains' unique habitats.

To fill knowledge gaps and enhance our understanding of Sambar ecology in this unique and sensitive habitat setting of wet patana, grasslands, and montane cloud forests within HPNP, a detailed study was conducted, including the

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assessment of population size, population structure, and ecological impacts of Sambar on the ecosystems in HPNP with the following objectives:

- i. To study the social organization and age structure of Sambar (*Rusa unicolor unicolor*) in Horton Plains National Park
 - a. Understanding the antler cycle of the male Sambar population
 - b. Estimation of the rate of recruitment through births in the Sambar population.
- ii. To determine the population dynamics of Sambar in HPNP.
- iii. To survey the availability of preferred grasses for Sambar in HPNP and assess their diet quality.
- iv. To study the impact of Sambar on the woody vegetation of HPNP.

II. METHODOLOGY

From 2017 to 2021, an extensive four-year research project was conducted in Horton Plains National Park (HPNP) to study the ecological dynamics and impact of Sambar deer. The study included diurnal and nocturnal observations across various climatic conditions, focusing on individual and social group behaviors. Detailed records were maintained on gender distinctions and physical attributes, with special attention given to calves up to one year old. A comparative analysis with captive populations at Dehiwala and Pinnawala National Zoological Gardens provided insights into morphological variations.

Over eighteen months (January 2018 to June 2019), the study investigated antler cycles and morphological variations within the Sambar population. Preliminary observations familiarized researchers with Sambar behavior and antler stages. Antler growth categories were defined, and fecal samples from specific males were collected for hormonal analysis using radioimmunoassay (RIA). This analysis correlated testosterone levels with antler stages, deepening the understanding of physiological processes.

Line transect sampling and DISTANCE 7.3 software were used to estimate Sambar density and abundance in HPNP's

grasslands. Social behaviors, such as sparring and mating, were documented, as well as calf recruitment. This comprehensive methodology integrated field observations, hormonal analysis, and statistical modeling to provide a holistic understanding of Sambar ecology and behavior in HPNP. The study also assessed the seasonality and nutritional aspects of preferred fodder grasses. Field observations and grass sample collections were conducted to analyze palatability and nutritional composition. Laboratory analyses determined proximate composition, organic matter digestibility, and metabolizable energy content, with statistical tests such as one-way ANOVA and the Friedman test assessing seasonal variations.

Satellite imagery and ground-based field surveys were utilized to assess vegetation changes, focusing on *Pennisetum* grass patches. Classification of satellite images and ground-truthing identified vegetation cover patterns and quantified changes in *Pennisetum* patches. Detailed vegetation sampling involved transects and subplots to represent forest patches, identifying woody species and recording bark damage. Visualization techniques, such as bubble graphs, were employed to represent relationships among variables related to tree health and bark damage intensity.

Overall, the study utilized a multifaceted approach, combining field observations, laboratory analyses, and remote sensing to comprehensively assess the ecological impact of Sambar deer browsing on vegetation in HPNP.

III. RESULTS AND DISCUSSION

Understanding the social dynamics and life cycle of Sambar populations is crucial for effective wildlife management. In the grasslands of Horton Plains National Park (HPNP), Sambar exhibit complex social behaviors, forming large groups year-round, although group sizes fluctuate seasonally. These groups engage in activities such as mating, ruminating, and foraging, with movement patterns involving transitions between grasslands and forest patches. Solitary bucks and lactating hinds typically stay closer to forest edges, with slight variations in movement during the rainy season. Sequential entry into grasslands is observed, with adult bucks joining groups, and the average encounter rate in HPNP's grasslands is approximately nine Sambar per kilometer during the evenings.

Group composition varies, with solitary individuals often being young or elderly stags, while hinds with calves form social units. Lactating hinds diligently care for their calves, often settling them under tree shade or in bushes while they graze, maintaining regular vigilance. Calves display playful and curious behavior, traveling in groups with hinds. Visual identification of sex and growth stages is standardized and tested, achieving high accuracy rates, particularly for older calves.

The antler growth stages of Sambar in HPNP have been meticulously documented, revealing a correlation between fecal testosterone levels and antler stages, with peak testosterone levels coinciding with courtship behavior. The shedding of antlers to velvet stripping stages occurs predominantly from April to September, with breeding behavior peaking from September to December. Peak calving is observed from July to August, with a decline in newborn

calf survival as they age, contributing to a shift towards a female-biased population structure over time. Despite no clear breeding seasonality, July-August is identified as the peak calving season.

The ecological density of Sambar in HPNP's grasslands peaks annually from November to December but shows a gradual decline over the study period. This decline is reflected in estimates of Sambar abundance based on grassland areas, indicating a decreasing trend. Social group dynamics vary seasonally, with mixed groups prevalent in late months and sex-segregated groups more common from January to June. Mating behaviors, such as mounting and sparring, are primarily observed from September to December.

Changes in forest composition within HPNP are noted, with montane forest cover expanding into grasslands, facilitated by the encroachment of species like *Rhododendron arboreum* and dwarf bamboo (*Arundinaria densifolia*). Grass palatability studies suggest *Pennisetum* spp. as the preferred forage for Sambar, with significant differences in nutritional composition among grass species. Bark damage to woody species by Sambar is evident, impacting forest health and contributing to dieback phenomena, particularly in susceptible species like *Litsea longifolia* and *Psychotria nigra*. This underscores the intricate relationship between Sambar browsing behavior and forest ecosystems in HPNP, emphasizing the importance of holistic wildlife management strategies.

IV. CONCLUSIONS

The study investigates the social organization, antler cycle, recruitment, population dynamics, grass availability, diet quality, and ecological impacts of Sambar deer in Horton Plains National Park (HPNP), Sri Lanka. Key findings include that identifying the sex and growth stage of calves improves with age, reaching over 90% accuracy after five months. The antler cycle of males correlates with fecal testosterone levels. Neonatal survival is around 44%, with calving peaking from July to August. Despite a population decline since 2000, Sambar numbers remain stable, with a 2018 peak of 2002 ± 238.6 . Preferred grass species like *Pennisetum* spp. are declining, impacting diet quality and contributing to population dynamics. The study reveals significant bark damage to woody species by Sambar, particularly affecting vulnerable species like *Litsea longifolia*. These insights are crucial for understanding Sambar ecology and developing effective conservation strategies in HPNP.

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INSTITUTION WHERE RESEARCH WAS CARRIED OUT

Postgraduate Institute of Science, University of Peradeniya, Sri Lanka, Department of Wildlife Conservation in Sri Lanka for permission to conduct our study in Horton Plain National Park under the research permit number WL/3/2/10/18 and National Zoological Gardens, Dehiwala, Sri Lanka.

FEEDING, RANGING AND SEXUAL BEHAVIOUR OF FREE-RANGING *Macaca sinica* ssp. *aurifrons* (DUSKY TOQUE MACAQUE) INHABITING LOWER HANTHANA, SRI LANKA

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I. INTRODUCTION

Macaca sinica (toque macaque) is the only endemic Cercopithecine Old World monkey of the five existing nonhuman primates in Sri Lanka. Due to urbanization, deforestation, fragmentation and agricultural expansions in the country, they are well adapted for any form of human settlement. Studies have been extensively conducted on population ecology and behaviour, but most of them are four to five decades old. There are only a few studies in the 20th century, yet the observations are only for a short period.

Hence a comprehensive study has been carried out focusing on quantitative analysis of the selected behaviours of *M. sinica* inhabiting lower Hanthana Sri Lanka for consecutive 13 months along with a floristic survey to examine the ecological parameters. Selected behaviours were foraging, ranging and habitat use and sexual behaviour, concerning the ecological parameters, including troop

structure and composition, food availability and distribution and weather parameters.

The overall objective was achieved by following four specific objectives;

- To assess the dynamics of troop structure and the composition of *Macaca sinica aurifrons* in lower Hanthana, Sri Lanka
- To study the dietary diversity and food selection of *M. s. aurifrons* in lower Hanthana, Sri Lanka
- To discuss the ranging pattern and habitat use of *M. s. aurifrons* in lower Hanthana, Sri Lanka
- To examine the sexual behaviours of *M. s. aurifrons* in lower Hanthana, Sri Lanka

II. METHODOLOGY

Only two macaque troops (namely Temple troop and Ice troop) were censused and observed continuously from August 2020 to August 2021, inhabiting the lower Hanthana study site. Data were collected 3-5 days/month using the scan sampling method (Altmann, 1974) from dawn until dusk (0700 hour-1700 hour) using an ethogram. Each scan lasted for ≤ 3 minutes for every 10-minute interval (Vandercone et al., 2012). The center of the troop was estimated visually and stopped to record the location every 10 minutes (Fashing, 2001) using a handheld Global Positioning System receiver. A floristic survey was conducted every last week of the month in 20×20 m vegetation plots to calculate the Basal area, Morisita's index and Food Availability Index. Weather parameters were obtained from the Natural Resource Management Center Gannoruwa, Department of Agriculture, Peradeniya, Sri Lanka.

Minitab 17.0 and R (3.4.1) were used for the analysis of the dynamics of troop structure and composition. Diet breadth, Dietary niche breadth, Shannon Diversity Index and Forage ratios were calculated to study dietary diversity and food selection. Mean daily path length, home range and Kernel density estimation were calculated using ArcGIS 10.4 (©1999-2014 Esri Inc.) to evaluate? the ranging pattern and habitat use. The frequency of heterosexual and homosexual copulatory behaviour was calculated to examine the sexual behaviours.

III. RESULTS AND DISCUSSION

The average troop size was 47.46 ± 5.22 and 23.00 ± 1.41 respectively in Temple and Ice troop. Understanding the correlation among the troop members is a key insight for social organization in macaque societies. Thereby, a strong positive correlation was found between the number of adult males (X_1) and that of adult females (X_2) ($X_1 \cdot X_2 = 0.81$) and the number of adult females and that of infants (X_5) ($X_2 \cdot X_5 = 0.61$) in the correlation matrix of the troop composition. (Altmann., 1990) proposed that the number of females ultimately controls the number of males found in a group as found in the study. Simply, “primate males go where the females are”. There was a high positive correlation between X_1 , X_2 and X_5 (89.37%) forming a sub-unit within the troop in the component correlation coefficients. This high positive correlation was similar to the Japanese macaque (Takasaki and Masui, 1984).

Extreme dietary specialization is rare among macaques. Yet there is usually a predominant type of food by which they can be classified. *M. sinica* preferred to eat fruits (37.06%) over leaves (16.68%), seeds (12.11%), garbage (11.29%), flowers (8.66%), provided food (6.96%) and insects (5.13%). Hence, *M. sinica* was verified to be an omnivore that predominantly feeds on fruits similar to the findings of (Dittus, 1974). Overall dietary niche breadth was recorded as 4.82 (Temple troop= 5.17 and Ice troop= 4.08). Monthly variation in dietary niche breadth varied from 6.38 to 2.86 (mean monthly= 4.47). In contrast, monthly niche breadth was recorded as higher for the endemic colobine *S. vetulus* (6.42) in Kaludiapokuna Forest Reserve (Vandercone, 2011). Comparisons between diet composition between sex ($W= 89$ $p= 0.0151$) and age ($W= 74$ $p= 0.0211$) were significant. These intraspecific differences can be due to the availability of food resources, habitat quality depending upon foraging options, seasonal influences and digestive system and their requirement of food nutrients. A total of 117 species belonging to 43 families were recorded as food plants. Of these 16 plant species were recorded as preferred food plants. Preferred seasonal food items were *Mangifera indica* (Clumped in distribution), *Hevea brasiliensis* (Random), *Ceiba pentandra* (Uniform), *Artocarpus heterophyllus* (Uniform), and *Ficus* sp. (both random and uniform). Fallback food items were *Spathodea campanulata* (Clumped), *Neolitsea cassia* (Random), and *Bridelia retusa* (Clumped).

Study troops represent their semi-arboreal nature. Occurrences recorded of terrestrial dais in Temple troop (31.08%) were higher compared to Ice troop (27.54%) ($W= 210$ $p= 0.001$). Annual mean daily path length was recorded as $1,582.58 \pm 295.23$ m in the Temple troop and $1,314.41 \pm 436.70$ m in the Ice troop respectively. A significant difference was found in monthly daily path length between the two study troops ($W= 223$ $p= 0.015$). The annual home range of study troops was more or less similar and did not overlap. It was calculated as $397,511$ m^2 and $398,692$ m^2 for Temple and Ice troop respectively (Figure 1). The monthly home range has a significant size difference ($W= 208.5$ $p= 0.001$). The core area of both troops was surrounded by frequently and readily accessible feeding patches

composed of either natural food sources or artificial food sources (Figure 1).

M. sinica was engaged in both heterosexual (51.5%) and homosexual (48.5%) courtships (Figure 2). Penile erection and intromission were observed in same-sex engagements where ejaculations were recorded in a few cases. Multiple mounting was observed in heterosexual ($n= 3$) and homosexual courtships ($n= 1$). Male-male engagement in homosexual courtship was high, where only 2.81% of female-female courtships were recorded.

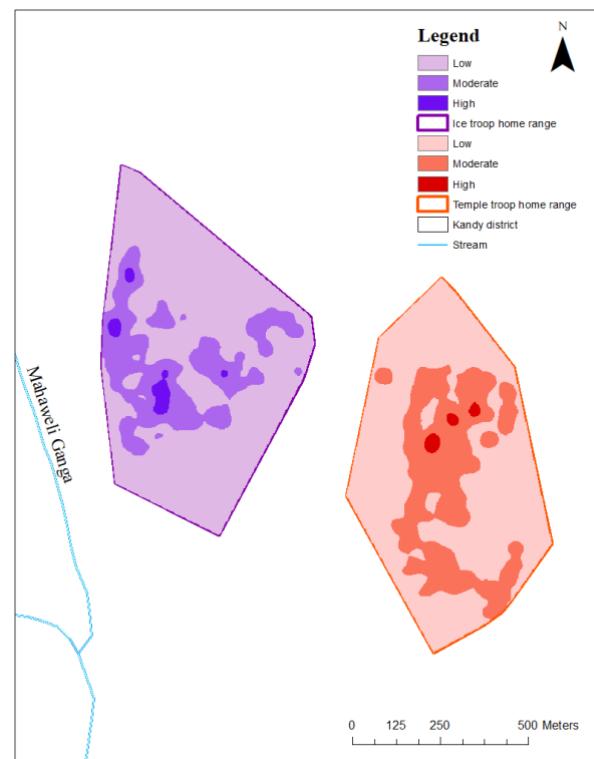


Fig. 1: Habitat use of two study troops in lower Hanthana

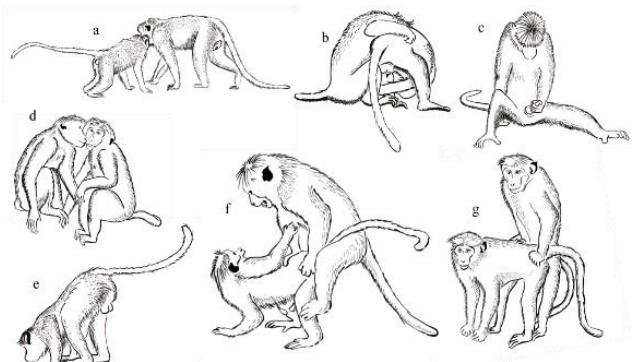


Fig. 2: Sexual behaviours observed; a: Bout, b: Opposite of manual erotic stimulation, c: Masturbation, d: Kiss, e: Penis display, f: Pause, g: Partial mount position

IV. CONCLUSIONS

M. sinica Statistical models can be developed concerning all factors to predict future troop size or variations. Such models can be useful to regulate the troop size of *M. sinica* for wildlife management purposes since they are considered both an Endangered species and an economical pest. Natural trends of the feeding behaviour of *M. sinica* would be challenging since the study site was subjected to heavy human alteration located in a secondary forest fragment surrounded by the University of Peradeniya. Thus, the findings of the study can be implied to mitigate the human-monkey conflicts: since observations were based in a habitat which was a combination of secondary forest patch and Use either SI (MKS) or CGS as primary units. (SI units are encouraged.) English units may be used as secondary units (in parentheses). human settlement. However, they were found to be omnivores that predominantly feed on fruits (37.06%), similar to other macaques in the region. Although *M. sinica* has been observed feeding on human food, no quantitative information on their habits was available to date. Hence this study found that they are relying on artificial food sources including, 11.29% of garbage and 6.96% of human-provided food, similar to other macaques in the region. Moreover, terrestriality of macaques has not been studied extensively and the present study documented the degree of terrestriality of *M. sinica* for the first time. The study can be applied to *M. sinica* populations dwelling in highly human-altered habitats including in urban cities, agricultural farmlands, fragmented forest patches or edges of forest reserve to understand the ranging pattern and habitat use to mitigate the conflicts arising from the negative interface between humans and monkeys. Forms of homosexual behaviour are newly documented in *M. sinica*. The functional context of this non-reproductive behaviour might be for pleasure-seeking, sociosexual aspects, greeting and reassurance, social bonding and an indication of close affection ties and practice for heterosexual activities. Future, research can be extended to investigating the role of kinship, female ovarian status and hormonal levels, social rank of both males and females, male and female preferences and inter-male competition.

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INSTITUTION WHERE RESEARCH WAS CARRIED OUT

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BEHAVIORAL RESPONSES OF COCONUT MITE, *Aceria guerreronis* AND VARIATION OF BIOCHEMICAL PROPERTIES OF COCONUT FRUITS OF SELECTED COCONUT VARIETIES IN RESPONSE TO COCONUT MITE INFESTATION

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I. INTRODUCTION

Coconut mite, *Aceria guerreronis* (Keifer) is one of the major pests of coconut in many countries causing severe yield losses including immature fruit drop, deformed fruits, empty fruits, and reduced fruit weight. Chemical and biological methods have been mostly used to control *A. guerreronis*.

In Sri Lanka, some commercial varieties are known to show the least damaged by *A. guerreronis* compared to other varieties. However, the reason for this observation is not known. Therefore, this study was done to reveal important biochemical information about coconut varieties of different susceptibility levels to coconut mite and their relationship with behavioral responses of *A. guerreronis* and its major predator, *Neoseiulus*.

baraki Athias-Henriot which would enable us to understand the drives of coconut mite resistance. This information can be used in selecting parental palms in future breeding for *A. guerreronis* tolerant/resistant coconut varieties.

The coconut varieties used in this study, Ran Thembili (RT), Gon Thembili (GT), Sri Lanka Green Dwarf (SLGD), Sri Lanka Yellow Dwarf (SLYD) and Sri Lanka Tall (SLT) are used as parent palms in producing hybrid coconut varieties in Sri Lanka.

The main objective of the current study is to identify biochemical profiles of *A. guerreronis* infested and uninfested fruits of different coconut varieties and understand their role in the host finding and oviposition behaviours/responses of *A. guerreronis* and *N. baraki*.

The following specific goals were set for the study to accomplish the main goal:

- i. To understand the searching behavior and reproductive performance of *A. guerreronis* on the selected coconut varieties.
- ii. To understand the searching behavior of *N. baraki* on the selected coconut varieties.
- iii. To determine changes in volatile organic compound profiles of the selected coconut varieties in response to infestation by *A. guerreronis*.
- iv. To determine changes in cuticular wax profiles of the selected coconut varieties in response to infestation by *A. guerreronis*.
- v. To determine changes in polyphenol profiles of the selected coconut varieties in response to infestation by *A. guerreronis*.

II. METHODOLOGY

Coconut fruits of RT, GT and SLT were collected from the palms located at the Bandirippuwa Research Centre of the Coconut Research Institute of Sri Lanka (CRISL) whereas coconut fruits of SLGD and SLYD were collected from the palms located at the Ambakelle Genetic Resource Centre of the CRISL, both are situated in Puttalam district of the North

Western Province of Sri Lanka. All coconut fruits used in the experiments were of 3-month-old after the opening of the inflorescence.

A. Searching behaviour of *A. guerreronis* on selected coconut varieties

The experimental setup that was used in the experiment was a modification of the method described by (Melo, et al., 2014). The setup consisted of a five-armed runway cut from a polyacrylic sheet (arena). Each arena was placed afloat in a water-filled Petri dish (9 cm diameter). Each arm of the arena was 3 cm long. *A. guerreronis* females were released at the center of the arena. Epidermal discs of 7 mm in diameter and 10 mm in height of tested coconut varieties were used as odor sources and kept at the distal ends of each arm. After confirming the unidirectionality of the setup, sixty adult female *A. guerreronis* of unknown age were transferred to the center of the arena and kept in the dark at 27 °C temperature and 70–80% relative humidity. The experiment was replicated 5 times. Number of coconut mites which had reached the distal end of each arm was recorded 3 hours after introduction of the coconut mites to the setup. The data were analyzed using Replicated G-test (Sokal and Rohlf, 2024).

B. Reproductive preference of *A. guerreronis* on selected coconut varieties

Washed and air-dried fruits of tested varieties, having perianth removed were wrapped individually with Cling Wrap, with a drop of paraffin wax underneath to form a narrow space between the wrap and the fruit surface. This rearing space was made on the meristematic region which is usually the feeding site of *A. guerreronis*. Thirty adult female *A. guerreronis* were transferred into the above space, through a tiny hole made in the cling wrap. The hole was later sealed with paraffin wax. Samples were kept in an incubator at 27 °C temperature. The first 24 hours of the study period was considered the conditioning period. Therefore, eggs present on the experimental setup within the first 24 hours after the introduction of *A. guerreronis* were discarded from the analysis. After the conditioning period, the number of live females and the number of eggs in the rearing space were counted at 24-hour intervals over 48 hours using a stereo-zoom microscope (Optika SZM-LED2, magnification: 10 x 45). The total number of eggs laid by coconut mites during the 48 hours was divided by the mean number of live *A. guerreronis* observed within the same timeframe to calculate the mean number of eggs laid by an individual mite. Analysis of Variance (ANOVA) and Post hoc mean comparisons test were done using Minitab software version 17.1.0.

C. Searching behavior of *N. baraki* on selected coconut varieties

A release-recapture method was used. Fifty adult female *N. baraki* aged 3-7 days since their last molt were kept starved for 6 hours and introduced in a Petri dish (diameter 3.5 cm) at the center of the arena specifically created for this experiment which was made up of a pentagonal black plastic sheet of 22 cm side, pasted on a plastic tray of 35 × 45 cm in size and kept inside a dark box at 27 °C temperature and 70–80% relative humidity for 1.5 hours before recaptured. The

periphery of the plastic sheet was marked with insect glue (Stikem, manufactured by ChemTica Internationals) to prevent the predators from escaping. A whole coconut fruit from each tested variety was kept at each corner as an odour source. Fruits were destructively sampled 1.5 hours after introducing *N. baraki* and the number of *N. baraki* on each fruit was counted. This experiment was carried out using 5 replicates. The data were analyzed using a Replicated G-test (Sokal and Rohlf, 2024).

D. Biochemical analysis of fruits of selected coconut varieties

Collection of volatile organic compounds from the fruits of *A. guerreronis* infested and uninjected nuts of tested coconut varieties were done separately using the dynamic head space sampling technique (Kumara, 2015). Each variety was triplicated. The volatile organic compounds of each sample were directed to be adsorbed into the PORAPAK-Q sorbent tube (ORBO™ 1103 PORAPAK™ – Q (50/80), 150/75 mg) for 6 hours. The adsorbed volatile organic compounds were eluted into dichloromethane and subjected to GC-MS analysis by using Thermo Fisher Scientific™ Trace 1300 GC/MS system with an auto-injector AI 1310 Thermo scientific, equipped with HP-5MS phenyl silox capillary GC column (Agilent Technologies, length 30 m; internal diameter - 0.25 mm; film thickness-0.25 µm). Mass spectral data was statistically analyzed by using the software MetaboAnalyst 5.0.

Cuticular wax of fruit samples used in the volatile collection described above was extracted to 100 mL of Chloroform (analytical grade) and evaporated to dryness. They were resuspended in chloroform to prepare wax samples of known concentrations. An aliquots equivalent to 1 mg of wax were derivatized by adding 300 µL pyridine and 300 µL BSTFA+TMCS (99:1) mixture. The derivatized wax was resuspended in 200 µL chloroform and was subjected to GC-MS analysis using Thermo Fisher Scientific™ Trace 1300 GC/MS system equipped with an auto-injector AI 1310 Thermo scientific equipped with HP-5MS phenyl silox capillary GC column (Agilent Technologies, length-30 m; inner diameter-0.25 mm; film thickness-0.25 µm). Mass spectral data was statistically analyzed by using the software MetaboAnalyst 5.0.

The epidermis (meristematic region) of coconut fruits used in cuticular wax extraction described above was blended with 150 mL of Methanol (analytical grade) to extract polyphenol compounds and filtered. The filtrate was subjected to HPLC using Agilent 1260 Infinity II system (Agilent Technologies), consisting of a diode array detector (WR G7115A) and equipped with a SUPELCOSIL™ LC-18 (Sigma-Aldrich Chemie GmbH, Octadecyl phase, 5 µm particle size, length x internal diameter: 25 cm x 4.6 mm) column and 2 solvent systems namely A and B. The composition of solvent system A was Acetonitrile (90): Acetic Acid (20): 20 mg/ml EDTA (2): Deionized water (888). The composition of solvent system B was Acetonitrile (400): Acetic Acid (10): 20 mg/mL EDTA (1): Deionized water (89). Compounds were detected at 278 nm wavelength.

The chromatography data was statistically analyzed by using MetaboAnalyst 5.0 software.

III. RESULTS AND DISCUSSION

Out of 300 *A. guerreronis* introduced in 5 replicates in the study to assess the searching behavior of them when they were offered different coconut varieties, 62–85% of coconut mites were recaptured on the setup out of which 76–95% were found on arms of the setup. There were no significant differences among replicates ($GH=11.58$; $df=16$; $p=0.77$) and therefore pooled data could be subjected to G-test. A uniform distribution of *A. guerreronis* on the five arms of the setup was observed 3 hours after the release ($GP = 1.74$; $df = 4$; $p = 0.78$). The results provided no evidence to support the fact that *A. guerreronis* has a particular preference or avoidance towards any of the tested varieties. In Sri Lanka, *A. guerreronis* was first reported in 1998. Therefore, given that the short period of time of the co-existence between *A. guerreronis* and the coconut varieties, it is unlikely that the *A. guerreronis* has developed the ability to perceive the differences of volatile cues from different coconut varieties.

The highest mean eggs per mite during 48 hours period (4.07) was observed on the variety GT whereas the lowest mean eggs per mite during 48 hours period (1.02) was observed on the variety SLYD. Although the varieties RT, GT and SLYD have been previously identified as less damaged by *A. guerreronis* (Perera, 2014) and their populations have been low on RT, GT and SLYD (Aratchige and Perera, unpublished data). In the present study, the egg production on RT and GT was higher than the other varieties tested. The possible reason for this difference could be the fact that in previous studies, the damage levels of the mite populations observed were results of *A. guerreronis* infestations over a long period of time whereas in the current study, the oviposition rate was observed for 48 hours only. However, the low level of damage observed on SLYD by (Perera, 2014) is supported by the low number of eggs on the same variety as observed in this study. In a separate study, it was observed that the population of *A. guerreronis* on immature fruits, damage initiation of the fourth bunch and harvest records were lowest in a hybrid coconut variety, Sri Lanka. Yellow Dwarf × Sri Lanka Tall in which the mother palm is SLYD (Perera, 2014).

Out of 250 *N. baraki* introduced, 60-84% could be recaptured on the setup when they were offered with fruits of different coconut varieties. There was no heterogeneity among replicates ($GH=19.76$; $df=16$; $p=0.23$). Therefore, pooled data was subjected to the Goodness of Fit test. An invariable distribution of *N. baraki* was observed among five different odour sources, 1.5 hours after they were released ($GP=4.94$; $df=4$; $p=0.29$). The results of this study produced no evidence that the *N. baraki* has a particular preference for or aversion to any of the tested varieties. With this result alone, it is impossible to conclude that the volatile profiles of tested varieties are similar. But, it can be hypothesized that *N. baraki* might respond differently to tested coconut varieties if *A. guerreronis* infested coconut fruits are offered as odour sources.

In volatile organic samples, there were 59 compounds detected, but only 26 were identified by the NIST/EPA/NIH Mass Spectral Library. The principle component analysis revealed some variety specific features in volatile organic compound samples. Volatiles, unknown 1 and octane were almost absent in the VOC profiles of variety GT irrespective of coconut mite infestation. Significant changes observed in the relative abundance of 1,2-Benzothiazole, α,α -dimethylbenzenemethanol, 10-Methylnonadecane, Acetophenone, 3-Carene, D-Limonene and I-Menthone are likely to reveal a herbivore induced response by the host plant because 1,2-Benzothiazole, α,α -dimethylbenzenemethanol, 10-Methylnonadecane, Acetophenone, 3-Carene levels are reduced in GT, RT and SLT when *A. guerreronis* is infested those varieties. D-Limonene concentration increased significantly in all the tested coconut varieties except SLGD whereas I-Menthone increased in GT and SLT in response to coconut mite infestation. This observation suggested these responses to be variety specific. However, it is worthwhile to test those compounds by using an olfactometer setup on both *A. guerreronis* and *N. baraki* to reveal their response to those compounds.

The cuticular wax profile consisted of 99 compounds of interest. In the current study, lupeol showed a drop in abundance upon coconut mite infestation of coconut varieties SLGD and SLT. SLT is expected to be susceptible to *A. guerreronis* as hybrid varieties of SLT (hybrid Sri Lanka Tall × Sri Lanka Tall) were found to be susceptible to coconut mite [4]. SLGD was known to be susceptible to coconut mite infestation (Aratchige, N.S. and Perera, S.A.C.N., unpublished), therefore lupeol can be suspected to have some interaction with the susceptibility of SLGD and SLT. This observation along with the elevated abundances of compounds having retention times of 34.93, 38.16, 41.76 and 42.40 in SLGD and SLT might indicate an herbivore induced activity which ultimately led both the varieties to be more susceptible towards coconut mite infestation. But none of the results obtained in behavioral studies supported this fact. The elevated abundances of compounds having retention times 34.55 and 41.31 in RT and GT upon coconut mite infestation are contrary to the results of SLGD, This finding might be interesting as it supports the field observations of these three coconut varieties in terms of susceptibility and resistance (SLGD – susceptible variety; RT and GT – resistant varieties).

The Polyphenol profile consisted of 41 compounds of interest. In the current study, increased concentrations of compounds having retention times of 15.50, 12.95, 7.73 and 13.94 over coconut mite infestation of SLYD when all other varieties showed decreased concentrations of the same compounds upon coconut mite infestation seems interesting as it provides clues of induced defensive activity of polyphenols which might explain the field-observed resistance of SLYD towards coconut mite infestation as well as the minimum oviposition rate of *A. guerreronis* observed in reproductive studies. Those compounds should have some degree of negative effect on coconut mite either by being toxic to them or unfavourable for feeding in order for the aforementioned argument to be satisfied.

IV. CONCLUSIONS

A. guerreronis and *N. baraki* seemed not to show any interest or avoidance on given odour sources under the conditions provided. SLYD's tolerance recorded previously was confirmed by the lowest oviposition rate of *A. guerreronis* on SLYD under the laboratory conditions provided. These observations seemed to have a relationship with herbivore induced elevation of certain polyphenols. Volatile, cuticular wax and polyphenol profiles of young coconut fruits are revealed for the first time. Some variety specific changes in these profiles upon coconut mite infestation are apparent. The rearing arena used in the evaluation of the reproductive preference of *A. guerreronis* is a newly developed approach that provides a habitat almost identical to their natural habitat and would be promising for future studies requiring *A. guerreronis* to breed.

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1. Behavioral studies of *A. guerreronis* and *N. baraki* were carried out at the Crop Protection Division of Coconut Research Institute, Lunuwila, Sri Lanka.
2. Biochemical analysis of coconut fruits was carried out at the Herbal Technology Section of Industrial Technology Institute, Malabe, Sri Lanka.

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