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RESEARCH

Volume 7

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Cover Image: With the pandemic behind us and new global challenges ahead of us, empowering the next generation with the knowledge, skills, and attitudes, they need to succeed has become a vital component of the progress of humankind. Universities strive to create a technologically skilled workforce to excel in the fast-moving contemporary world. At the same time, graduates must have the necessary human elements like communication, empathy, and emotional intelligence to make their leadership count and their impact felt in a society that yearns for visionary leadership. The challenge of the decade in Science Education would be finding the perfect balance between the aspects of science and humanities through student-centred learning. The cover image of a floating book at a library by Jaredd Craig signifies the need for finding this perfect balance.

Cover Design: Dr. A.K. Amarasinghe, Faculty of Science, University of Peradeniya.

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PGIS POSTGRADUATE RESEARCH HIGHLIGHTS 2022

THE MESSAGE FROM THE DIRECTOR

It is with great pleasure and pride that I write this message for the 7th volume of the Research Highlights of the Postgraduate Institute of Science (PGIS). Publishing the Research Highlights of PGIS has been introduced to show the outcome of research work conducted by our students and their supervisors to the scientific community and society. Out of the research degrees awarded in the recent past, some of their research works have been incorporated into this year's volume.

When young scientists are striving to establish themselves as scientists, publishing their research may be helpful to their reputation and recognition advancement, which can help them progress up the career ladder. It is unethical for some research leaders to seek credit for the work of junior researchers. Despite being a bad habit, it has occurred all over the world. The "Research Highlight Brochure," which is issued in conjunction with the PGIS research congress-2022, was created by PGIS in an effort to assist new researchers to establish their own academic profiles with the guidance of their supervisors. It should assist young scientists in establishing themselves and gaining experience in the concise compilation of their research work. Some researchers have also received awards for their remarkable research, and many of their findings have already been published in respected journals.

The team has done an excellent job from preparation to publication, and I respectfully appreciate the dedicated service of Prof. Champika. V. Hettiarachchi as the Editor-in-Chief of this publication and the members of her review group for their valued contribution. I want to sincerely thank all of the M.Phil. and PhD. graduates of the PGIS as well as their supervisors for providing summaries of their important research and giving their permission to publish it.

Prof. H.M.T.G.A. Pitawala,
Director
Postgraduate Institute of Science, University of Peradeniya

THE MESSAGE OF THE EDITOR-IN-CHIEF

It gives me great pleasure and pride to write a few words on the completion of the Research Highlights - 2022. This is the 7th volume in a series which was initiated in 2016. This volume is compiled with the research outputs of five Ph.D., seven M.Phil. and four M.Sc. (SLQF 10) Degrees out of 46 research degrees completed in the year 2021. I must congratulate all the postgraduates who received their degrees and their respective supervisors for contributing to disseminate the valuable research findings to the scientific world through this volume.

Research is the key component in postgraduate studies and contribute immensely for the development of sciences and thereby the entire world. In this year, it was decided to publish the Research Highlights - 2022 only as an e-book due to the prevailing conditions in the country. However, it would provide us a greater opportunity to grasp a wider readership globally of Research Highlights - 2022. Hence, I strongly believe that this publication would be a valuable handbook for local or international researchers who wish to conduct collaborative research with local scientists as well as with the PGIS.

I take this opportunity to especially thank all the researchers and their supervisors who submitted their research highlights by meeting the deadline for this year's publication. Untiring dedication and support that I received from the Editorial Team of the Research Highlights - 2022 are greatly appreciated, without their contribution this would not be at this success. I must express my thanks to Dr. Ashwini Amarasinghe for preparing an appropriate cover page for the current volume. Finally, my sincere thanks go Prof. H.M.T.G.A. Pitawala, Director PGIS and Prof. H.M.S.P. Madawala, Chairperson of the RESCON-2022 for their valuable guidance and encouragements throughout the preparation period of this document.

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PHYSIOLOGICAL CHARACTERIZATION AND ASSESSMENT OF GENETIC DIVERSITY OF RHIZOBIAL STRAINS IN *Clitoria ternatea L.* IN ANURADHAPURA DISTRICT, SRI LANKA

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Rhizobia are prokaryotes that are capable of reducing inactive atmospheric nitrogen into ammonia through a process known as Biological Nitrogen Fixation (BNF) (Santi *et al*., 2013). This process of BNF fulfills the nitrogen requirement of the plant.

Clitoria ternatea is a leguminous plant that hosts the rhizobia and fixes the atmospheric nitrogen through BNF. *C. ternatea* is abundant and naturally growing in the Anuradhapura district of Sri Lanka. These plants can be grown in extreme environmental conditions such as prolonged droughts and in extended rainy seasons. Hence, *C. ternatea* may harbor different stress-tolerant rhizobial strains which could be used for cross-inoculation of crop legume plants that are grown in stressful environments such as high salinity, high temperatures, extreme pH, and drought. When these stress-tolerant rhizobial strains are successfully cross-inoculated into the crop plants, they can enhance the process of atmospheric nitrogen fixation and fulfill the nitrogen requirement of the crop plant. Moreover, some studies showed that cross inoculation of rhizobia to the crops has increased the crop yield and plant growth than applying chemical fertilizers (Bhardwaj *et al*., 2014). However, the studies on rhizobia and *C. ternatea* symbiosis in the Anuradhapura district are limited.

The objectives of the present study were,

(I)   To isolate the stress-tolerant rhizobial strains from the root nodules of *C. ternatea* in the Anuradhapura district of Sri Lanka

(II) To assess the genetic diversity of the obtained stress-tolerant rhizobial strains in *C. ternatea.*

Methodology

The root nodules from the *C. ternatea* from randomly selected seven different locations (Anuradhapura urban, Medawachchiya, Kahatagasdigiliya, Thanthirimale, Mihintale, Thalawa, and Palugaswewa) of the Anuradhapura district were collected and rhizobia inhabiting in the root nodules were isolated in ½ Lupin agar medium. After 4-5 subcultures, 28 pure rhizobial colonies were obtained and the isolated rhizobia were grown in ½ Lupin broths, under different physiological conditions such as temperature (25 °C, 30 °C, 35 °C, 40 °C, and 45 °C), salinity (salinity was induced by adding different NaCl concentrations to the growth medium w/v, 0.1%, 1%, 1.5%, 2%, 2.5%, and 3%), pH value (3 - 9), and drought (the drought condition was induced by adding Polyethylene glycol-8000

(PEG) to the growth medium, w/v 0.1%, 0.2%, 3%, and 0.4%). Twelve stress-tolerant rhizobial isolates were selected and they were subjected to the combination of physiological studies (pH 8.0, temperature 36 °C, salinity 3%, and drought 0.4%). Then these 12 stress-tolerant rhizobial isolates were subjected to PCR based Enterobacterial Repetitive Intergenic Consensus (ERIC) profiling. A dendrogram was constructed from the obtained DNA banding pattern using the Average Linkage, Euclidean Distance method using the MINITAB 17.1.0 software.

Results and Discussion

Most of the rhizobial isolates were well-grown within the pH range of 5.0 to 8.0. Almost all the rhizobial isolates showed poor growth in the lower pH range of 3 - 4. However, the strain AP-2 (Anuradhapura urban site) showed a higher tolerance even at pH 3. AP-2 isolate also showed the highest growth at the pH 9.0 among all the other isolates. Furthermore, there was no clear observable pattern in the growth of 28 rhizobial isolates at different pH values. Generally, the optimum pH range for the growth of rhizobial strains is between 6.0 -7.0 (Somasegaran and Hoben, 2012). However, the most of the rhizobial colonies isolated in this study showed an optimum growth in the pH range of 5 to 8. The soil pH of the Anuradhapura district varies between 4.5 - 8.0 (Renuka and Senevirathne, 2017). Therefore, this suggests that the rhizobial colonies isolated from the Anuradhapura district are well adapted to the prevailing pH conditions in the soil.

The graphs of the growth of the twenty-eight rhizobial isolates at different NaCl concentrations did not highlight a common pattern. AP-2 isolate showed the highest tolerance at 3% NaCl concentration. Moreover, most of the rhizobial isolates showed comparatively higher growth even at 3% NaCl concentration. Anuradhapura district is in the dry zone where the temperature is high and the annual rainfall is low. This leads to elevated evaporation, eventually resulting in high saline soil. Thus, most of the rhizobial isolates tend to adapt to the prevailing salinity conditions.

The isolated 28 rhizobial isolates were grown at ½ Lupin broths at different temperatures ranging from 25 to 45 °C and the highest growth was observed at 30 and 35 °C. In the

Anuradhapura district the mean maximum temperature is ranging from 26.2 to 33.9 °C (Udupamunuwa *et al.*, 2020). Thus, the results clearly depict that the rhizobial strains are well adapted for the prevailing temperature condition. The isolate KH-3 (from the Khatagasdigiliya site) showed the highest survival at 45 °C.

Significant growth was shown by all twenty-eight rhizobial isolates under different PEG-induced drought conditions where the PEG concentration of the growth medium varies from 0.1% to 0.4%. Most of the strains showed the highest growth at 0.2% PEG concentration. Some isolates survived well even at the 0.4% PEG concentration. Anuradhapura district is located in the dry zone and it experiences high evaporation leading to water scarcity that introduces the drought conditions. Hence, results show that most isolates are well adapted to the prevailing drought condition in the Anuradhapura district.

The isolates which showed high tolerance to more than two extreme physiological conditions were subjected to the combination physiological test (figure 1). PG-1 (from the Palugaswewa site) showed the highest survival in the study of the combination of physiological conditions where it showed maximum tolerance at 0.4% PEG concentration and 3% NaCl concentration. However, it did not show high tolerance at 45 °C and pH 9.0. However, PG-1 showed high tolerance when grown at 35 °C and pH 8.0 separately. Thus, supporting the optimum growth of PG-1 is at the conditions used for the physiological characterization.
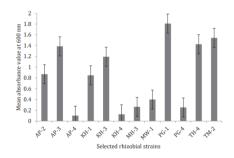

Figure 1: Growth of the 12 selected rhizobial strains in combination of different physiological conditions (pH=8.0, Salinity 3.0 %, Drought 0.4 % and incubated at 36 °C). (PG-1a , TM-2a , TH-4a , AP-3a , KH-3a,b,

AP-2a,b,c, KH-1a,b,c, MW-1b,c, MH-3b,c, PG-4b,c, KH-4c , AP-4c) at 600 nm of optical absorbance (Means denoted by same letters are not significantly different at p<0.05)

In the ERIC profile (figure 2), MW-1 and MH-3 were similar to each other at 100% similarity level. The next level was observed at 69.89% similarity where 11 clusters were obtained. However, there was no correlation between the strains and the geographical range as the same rhizobial strains were found in different geographical ranges.

Figure 2: The dendrogram with Average Linkage and Euclidean Distance showing the genetic relationship of 12 stress-tolerant rhizobial strains.

Conclusion
In this study, 12 rhizobial strains were identified and all showed high tolerance to extreme physiological conditions of temperature, salinity, drought, and pH. According to the dendrogram prepared using the ERIC profile, it was found that these strains are highly genetically diverse.

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Institutions where the research was carried out
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SAR STUDIES OF 6β-HYDROXYBETUNOLIC ACID AS ANTIMICROBIAL AGENT

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Resistance of bacteria to antibiotics is one of the major burning issues worldwide which requires urgent action. Hence, finding alternative antibacterial agents is a timely need. About one-third of the best-selling drugs in the world originated directly or indirectly from natural products (Newman and Cragg, 2016); hence, the use of plant secondary metabolites with vast structural diversity and bioactivities as drug lead compounds is seen as an alternative to overcome the current issue.

6β-Hydroxy-3-oxolup-20(29)-en-28-oic acid (6β-hydroxybetunolic acid), **1**, isolated from *Schumacheria castaneifolia*, a plant endemic to Sri Lanka has been reported to possess antibacterial activity against the Gram-positive strain of *Staphylococcus aureus* (*S. aureus*) with Minimum Inhibition Concentration (MIC) of 50 mg L^{-1} (Bandara, 2014). It consists of main structural differences at C3 (carbonyl group) and C6 (hydroxyl group) compared to other common naturally occurring lupane-triterpenoids such as betulinic acid, lupeol, and betulin (figure 1).

The objectives of the present study were,
(I) To evaluate the antibacterial potential of 6β-hydroxybetunolic acid against selected common pathogenic Gram (+) and Gram (-) bacterial strains
(II) To test the combinational effect of 6β-hydroxybetunolic acid with oxacillin against *Staphylococcus aureus* and *methicillin-resistant S. aureus*
(III) To synthesize new semi-synthetic derivatives of 6β-hydroxy betunolic acid
(IV) To study the SAR of structural analogs of 6β-hydroxybetunolic acid as antimicrobial agents

Methodology
Determination of the antibacterial potential (MIC values) of 6β-hydroxybetunolic acid and its structural analogs was done using the broth microdilution method adapted from the guidelines set for clinical laboratories by the Clinical and Laboratory Standards Institute (CLSI, 2020) against the sixteen pathogenic

strains including seven Gram-positive bacterial strains (*Staphylococcus aureus* (ATCC 29213 and ATCC 25923), *Enterococcus faecalis* (ATCC 29212), and four methicillin-resistant *S.aureus*) and nine Gram-negative bacterial strains (*Escherichia coli* (ATCC 35218 and ATCC 25922), carbapenemase-producing *Klebsiella pneumoniae* (ATCC BAA 1705), carbapenemase non-producing *K. pneumoniae* (ATCC BAA 1706), *Pseudomonas aeruginosa* (ATCC 27853), and a meropenem-resistant *Acinetobacter* sp).

The synergistic, additive, or antagonistic effect of 6β-hydroxybetunolic acid in combination with oxacillin against *S. aureus* (ATCC 29213) and four methicillin-resistant *S. aureus* strains (MRSA 114, MRSA 124, MRSA 58, and MRSA 59) were determined by using checkerboard dilution test (Jain *et al.*, 2011).

Thirteen structural derivatives of 6β-hydroxybetunolic acid (**1**) were synthesized by modifying its main functional groups at C3, C6, C17, and C20 positions (figure 1) to obtain compounds **2**, **3**, **4**, **5**, **6**, **7-11**, **12** and **13** respectively (figure 1).

*7: CH$_2$C$_6$H$_5$, 8: CH$_2$CH$_2$Cl, 9: CH$_2$CHCH$_2$, 10: CH$_2$COC$_6$H$_5$, 11: CH$_2$CH$_2$CH$_3$

Figure 1: Structural analogs of 6β-hydroxybetunolic acid

Results and Discussion

Antibacterial activity was measured in terms of MIC using the broth microdilution method according to the guidelines set for clinical laboratories by CLSI. The resulting MIC values obtained against Gram-positive organisms and Gram-negative organisms were tabulated in Table 1.

Results revealed that 6β-hydroxybetunolic acid has significant antibacterial activity (MIC < 32 mg L^{-1}) against all the tested Gram- positive organisms. Based on the results, compound **5** was found to display improved antibacterial activity against *S. aureus* ATCC 29213 (16 mg L^{-1}) and MRSA 114 (8 mg L^{-1}).

Table 1: MIC values of 6β-hydroxybetunolic acid and its derivatives against tested organisms. Each MIC value represents the mode of triplicates

position changed	analogs	MIC values (mg L^{-1})							
		S. aureus (ATCC 29213)	*S. aureus* (ATCC 25923)	*E. faecalis*	MRSA 114	MRSA 124	MRSA 58		Gram-negative strains
-	1	32	16	32	32	32	32	16	>1024
C3	2	256	128	512	128	512	256	512	>1024
	3	128	128	256	128	128	128	128	>1024
	4	256	64	128	128	256	64	128	>1024
	5	16	16	64	8	256	256	128	>1024
C6	6	1024	128	256	128	128	64	64	>1024
C17	7-11	>1024	>1024	>1024	>1024	>1024	>1024	>1024	>1024
C20	12	512	512	256	1024	>1024	>1024	>1024	>1024
	13	256	256	128	1024	>1024	>1024	>1024	>1024

However, all the other modifications detrimentally affected the antibacterial activity against tested Gram-positive organisms. The most predominant structural change on antibacterial activity was observed with the ester modifications at C17. However, MIC values of 6β-hydroxybetunolic acid and all its derivatives against all the Gram-negative organisms had higher values than the tested concentration range. It is anticipated that this is due to the cell wall difference between the two groups.

Checkerboard assay was performed to study the synergistic activity between the standard antibiotic oxacillin and the 6β-hydroxybetunolic acid on *S. aureus* (ATCC 29213), MRSA 114, 124, 58 and 59 and results are tabulated in Table 2.

Based on the results, when in the presence of 6β-hydroxybetunolic acid at its ¼ MIC value, the MIC of oxacillin was reduced by as much as 4-fold, from 0.25 to 0.06 mg L^{-1} against *S. aureus* ATCC 29213 and indicated the presence of synergism. It is interesting to note that the MIC of oxacillin was reduced by 32 folds (from 16 to 0.5 mg L^{-1}) against MRSA 114, which made the MIC of oxacillin to be less than the sensitive breakpoint, 4 fold (from 16 to 4 mg L^{-1}) against MRSA 59 and two fold (from 16 to 8 mg L^{-1}) against MRSA 58 when in the presence of 6β- hydroxybetunolic acid at its half MIC value; however, calculated FIC indices for 6β- hydroxybetunolic acid in combination with

oxacillin against MRSA strains indicated only the presence of additive effects.

Conclusion

6β-hydroxybetunolic acid showed significant antibacterial activity (MIC < 32 mg L^{-1}) only against the Gram-positive strains; hence, it is concluded that the antibacterial activity of 6β-hydroxybetunolic acid is predominantly depending on the cell wall difference of the bacteria. Derivative with acetyl group at C-3 has increased its antibacterial activity in one-fold against both *S. aureus* (ATCC 29213 and ATCC 25923) and two-fold against MRSA 114. These results concluded that the change of the carbonyl group at C-3 has a moderate effect on the antibacterial activity against Gram-positive organisms; however, the presence of the hydroxyl group at C-6 and olefin group between C-20 and C-29 is important for the antibacterial activity showed by 6β-hydroxybetunolic acid against Gram-positive organisms. Further, the presence of the carboxylic acid group at C-17 may be an essential factor for the shown antibacterial activity of this compound. Moreover, 6β-hydroxybetunolic acid has a synergistic effect with Oxacillin against *S. aureus* and an additive effect against all the tested MRSA.

Source of Funding

Table 2: The summary of the results of checkerboard assay

Organism	MIC (mg L^{-1})				Fractional inhibition concentration (FIC)		FIC index	Decision
	Alone		Combination					
	Ox	6β	Ox	6β	Ox	6β		
S. aureus	0.25	32	0.06	8	0.24	0.25	0.49	Synergism
MRSA 114	16	32	0.5	16	0.03	0.5	0.53	Additive
MRSA 124	16	32	16	32	1	1	2	Additive
MRSA 58	16	32	8	16	0.5	0.5	1	Additive
MRSA 59	16	16	4	8	0.25	0.5	0.75	Additive

Note: Each MIC value represents the mode of triplicates
Ox:Oxacillin; 6β:6β-hydroxybetunolic acid

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GRAPHITE AS A POTENTIAL SUBSTRATE FOR NANO ZERO VALENT IRON STABILIZATION AND NITRATE DESTRUCTION

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In the past decade, acute water stress throughout the world has been increasing due to the rapid population growth and over-exploitation of groundwater resources. According to the United Nations (UN) predictions, up to 5.7 billion people could be suffered from water scarcity by 2050 while the majority continue to live under water stress (Ahmed *et al*., 2020). Therefore, currently, there is a critical need to identify and develop reliable solutions that ensure sustainability of resources by understanding the energy-water nexus. To address most of these problems, United Nations Sustainable Development Goals 6 (UN SDG-06) entails safe drinking water and sanitation for the whole world by 2030.

Due to the sparse population density, conventional treatment systems with piped networks cannot be installed in lower-income countries. The community-based water supply schemes are introduced as a viable option to meet the water needs of areas with limited success. However, decentralized water treatment plants are the right choice for rural communities with sparse population densities (Shen and Schäfer, 2014). Therefore, to desalinate brackish water, most of the countries currently rely on well-established technologies such as reverse osmosis (RO) and electrodialysis (ED).

Fluoride is a primary water quality standard that has enforceable values of 1.5 mg L^{-1} according to the World Health Organization (WHO). However, the water hardness is a secondary water quality standard which is non-enforceable. High hardness (and total dissolved solids) renders water unpalatable, which restricts it consumption. Therefore, improvement of water palatability is essential before treating excess fluoride or other bioactive solutes present in traces.

To remediate contaminated groundwater, zero-valent iron (nZVI) has been used since the early 1990s (Gillham and O'Hannesin, 1994). However, nZVI has received much

more attention in the past few decades due to its key properties. Hence, a higher reactivity (Mikhak *et al*., 2017) towards a broad range of contaminants including halogenated compounds, polycyclic aromatic hydrocarbons, fluoride, nitrate, phosphate like inorganic and organic pollutants is due to its reduced size (Chen *et al*., 2011). nZVI particles can be directly injected into the sub-surfaces, which is an effective and promising technology for *in-situ* groundwater remediation. Additionally, its application does not require excavation as highly concentrated nZVI slurries are directly injected underground at or near the source of contamination (Lefevre *et al*., 2016). Apart from these properties, being a non-toxic, inexpensive and environmentally compatible material, nZVI particles follow the maximal tenets of green chemistry (Wen *et al*., 2014).

The objectives of the present study were,

(I) To give a significant value for Sri Lankan graphite. This research focuses on fabrication and characterization of stable and well-dispersed nano zero valent iron on reduced graphene oxide (rGO-nZVI) using polyphenols extracted from green tea (hereafter rGO-nZVI-P) and sodium borohydride (rGO-nZVI-B).

(II) To identify reduction efficiency of rGO-nZVI–P against rGO-nZVI-B using aqueous nitrate as an index ion.

Methodology
Morphology and structural characterization
The rGO-nZVI composites were characterized using spectroscopic evidences. The morphology of the rGO-nZVI composite materials were identified with transmission electron microscopy. X-ray diffractograms of rGO-nZVI composites were obtained for phase identification and particle size estimation. Raman measurements, Fourier transformed infrared spectra and X-ray photoelectron spectroscopic (XPS) evidences were used to postulate nitrate reduction pathways.
Nitrate reduction by rGO-nZVI composites
To reduce nitrate in water, rGO-nZVI composites were used and the experimental procedures were documented elsewhere (Ratnayake *et al*., 2017). In a typical experimental setup, a 200 mL batch solution

was prepared in 0.01 mol dm^{-3} NaCl using 5 g L^{-1} rGO-nZVI composites at pH ~5.6 and 25 °C. Ar was continuously purged and batch reactor was water-jacketed to achieve the desired temperature (within ± 0.01 ℃). At a given point, the solution was spiked with 50 mg L^{-1} nitrate, and at pre-defined times and a known volume of suspension was filtered to detect nitrate and nitrite by ion chromatography. All measurements were carried out in triplicate and empirical rates of nitrate reduction by rGO-nZVI composites were developed from the experimental data.

Following the same procedure, well water sample in Nettiyagama village (Sri Lanka); which is contaminated with nitrate (94 mg L^{-1} NO$_3^-$) was used to evaluate the efficiency of rGO-nZVI catalysts for nitrate destruction.

Results and Discussion
The X-ray diffraction peaks confirm the presence of α-Fe (0) BCC phase in the core and the conversion of graphene oxide (GO) into rGO by exfoliation. With a layer thickness around 2–7 nm the shell structure is composed of mixed iron oxides/ hydroxides.

Figure 1: (a) Raman and (b) FTIR spectra of graphite, GO and rGO-nZVI composites.

According to the TEM images, the graphene sheets form stable configurations as corrugation, bending, and folding. Most nZVI particulates are spherical with diameters in the range ~4 to ~15 nm, which are well dispersed within rGO layers, and inhibits rapid oxidation of Fe(0) and particles agglomeration. The Raman spectrum of nZVI is virtually featureless. However, in rGO-nZVI-B, the band at 2680 cm^{-1} indicates mono-layering in graphene structure, whereas a multi-layering structure was observed in rGO-nZVI-P.

(a) **(b)** **(c)**

Figure 2: (a) X-ray diffractograms of rGO – nZVI-B and rGO – nZVI-P and (b) Corrugation and folding morphology of rGO and (c) nZVI core shells and oxidised particulates.

The absence of IR band at 1724 cm^{-1} in both composite spectra confirms the conversion of GO → rGO. Additionally, presence of Fe$_x$O$_y$ phase on graphene sheets were further confirmed with the band at 598 cm^{-1}. All these spectral evidences confirmed the association of Fe (0) on rGO matrix via π–π framework.

Figure 3: A postulated model for the association of Fe (0) with rGO.

At around pH 5.6, the rGO-nZVI-P shows enhanced reactivity for nitrate reduction compared to rGO-nZVI-B. Accounting over 95% of N-mass balance, ammonium ion is the dominant nitrate reduction product by rGO-nZVI. Initially, nitrite and nitrogen oxides are present in small quantities, however, no nitrite accumulation is shown later. Nitrate reduction does not occur in homogeneous solution in the presence of Fe(II), and it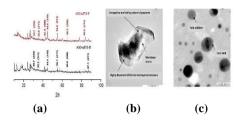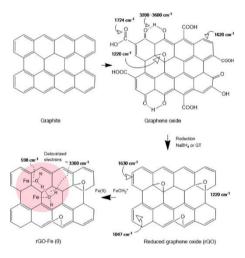 is essentially surface-mediated. The surface of rGO-nZVI composites is positively charged (pH$_{zpc}$ values of rGO-nZVI-P and rGO-nZVI-B are 6.2 and 6.9, respectively) when pH ~5.6, and NO$_3^-$ forms an outer-sphere surface complex without a ligand exchange. Additionally, the transfer of electrons occurs readily to nitrate ions via surface attached OH$^-$ conduits. The experimental data suggest ammonium as a major product, while N$_2$, NO and NO$_2^-$ present in the system in traces.

The development of a mechanistic model to account for nitrate reduction kinetics is quite challenging because of multi-electron transfer routes of rGO-nZVI composites. An empirical kinetic model was developed to quantify NO$_3^-$ reduction by rGO-nZVI composites using the initial rate determination method. At pH 5.6, the nitrate reduction by nZVI composites follows second-order kinetics.

In a batch experiment, rGO-nZVI composites were used to destruct nitrate in a well water sample used for human consumption which exceeds concentration of nitrate than the WHO limit. Over 55% nitrate in the well water can be eliminated by rGO-nZVI-P treatment and additionally over 40% Total Dissolved Solids (TDS) reduction in water can also be achieved. However, only 43% of nitrate was destructed with rGO-nZVI-B and there was no decrease in TDS. The divalent cations in contaminated water seem to complex with polyphenolic compounds present in rGO-nZVI-P. Therefore, rGO-nZVI-P has a promise in drinking water treatment due to its ability to reduce nitrate and TDS in natural water concurrently.

Conclusion
The stability of nZVI was enhanced by fabricating it on rGO matrix using green tea derived polyphenols as a reductant. The incorporation of nZVI amidst rGO is believed via π–π stacking. Both composite materials destruct nitrate efficiently yielding ammonia as the main product (over 95 % of N-mass balance). Compared to rGO-nZVI-B, rGO-nZVI-P promise to reduce nitrate and TDS in natural water concurrently. Further research warrants into in-situ conversion of ammonium produced by nitrate reduction into chloramines for safe water disinfection.

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In silico INVESTIGATION OF COMPLEXATION BEHAVIOUR OF CYCLODEXTRINS

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Cyclodextrins (CDs) belong to the family of cage molecules; the core of their structure is composed of a three-dimensionally stable, relatively hydrophobic cavity that can trap or encapsulate other molecules forming inclusion complexes (Davis and Brewster, 2004). The α, β and γ-CDs, which consist of six, seven and eight glucopyranose units, are used extensively in pharmaceutical formulations as a functional excipient. The cavity sizes of all three CDs have been approximated using static structures, however, the actual size range of guests that CDs can accommodate, and the relative contributions of interactions involved in the complexation process (Faucci *et al*., 2002) have not been systematically studied. Historically, cyclodextrin-based formulations have been developed through trial-and-error, which is both time and resource-consuming, and moreover, does not enhance our understanding of the complexation.

Here, a novel approach was taken to measure the size range of the guest molecules that these CDs can accommodate and to find the important anchoring groups of guests that stabilise the β-CD complexes. Molecular dynamics computer simulations were carried out using spherical implicit continuum particles of different sizes to observe complex-forming behaviour and to determine the maximum size of the guest that they can hold. Later selected non- steroidal anti inflammatory drugs (NSAIDs) were used to examine the complex forming behaviour.

The objectives of the present study were,

(I) To measure the size range of the guest molecules that CDs can accommodate by varying the size of a generic probe particle devoid of any functional groups.

Encapsulation of drugs in host systems not only serves as an effective tool for delivery, but in some cases, is also known to increase their therapeutic efficacy.

(II) To study the effect of functional groups / anchoring atoms during the complexation of *β*-CD with NSAIDs

Methodology

size range of the guest molecules that CDs can accommodate

Continuum model allows the solid particle to be an idealised single interaction bead, which captures essential properties while maintaining computational efficiency. This approach provides advantages such as (1) elimination of varying geometries of the guest molecules, (2) ability of changing the sizes of particles without introducing different functional groups, and (3) consistent measurement of positioning of the guest particle.

i) Developing continuum model

Continuum model allows for smearing out site-site interactions, and determining the total interaction potential between the probe particle and an external atom. Figure 1 shows a graphical presentation of the developed model.
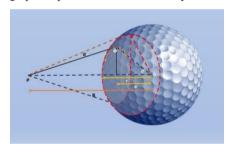

Figure 1: The total potential of an atom (at point P) with a solid particle (at origin O) can be found by integrating the site potential at a point (a, θ,) within the sphere. Here R is the distance between the outside atom and the centre of mass of the probe particle.

If a standard Lennard-Jones (LJ) potential is assumed for the site-site interaction, the integrated potential function is given by,

$$U(R; r) = \frac{16\pi\rho\epsilon\sigma^{12}r^3(5r^6 + 45r^4R^2 + 63r^2R^4 + 15R^6)}{45(R-r)^9(R+r)^9} - \frac{16\pi\rho\epsilon\sigma^6 r^3}{3(R-r)^3(R+r)^3}$$

Where the r is the radius, R is the separation, ρ is site-density and σ and ε are LJ parameters.

ii) Determining hydrophilicity

The behaviour of the particle is completely determined by the interaction potentials between the probe and surrounding molecules.

Based on its ε and σ values. After validation, the following values were assigned: hydrophilic particle ε = 0.4 kcal mol⁻¹, intermediate particle ε = 0.2 kcal mol⁻¹ and a (super) hydrophobic particle with ε ≈ 0.0 kcal mol⁻¹. Non-bonded interaction parameters were calculated using the Lorentz-Berthelot mixing rules.

Effect of functional groups / anchoring atoms on the complexation

i) Selection of drug molecules

The drug molecules were chosen to represent a range of molecular weights, functional groups and hydrophobicities. Nine NSAIDs (Aspirin, Ibuprofen, Piroxicam, Meloxicam, Ketoprofen, Indomethacin, Naproxen, Diclofenac, Acetaminophen) were studied concerning their complexation with *β*-cyclodextrins. A total of fifty different CD-Drug conformations were simulated for each. Binding energy, solvent accessible surface area (SASA) and contact maps were generated.

Simulations

All simulations were carried out using NAMD (Phillips *et al.,* 2005) 2.10 molecular dynamics software. System components were represented using the CHARMM (version 36) force field (Vanommeslaeghe and Mackerel, 2015).

Results and Discussion

Computer simulations are a useful tool to gain information about conformational dynamics, the energetics, and the complex-forming ability (Ivanov 2010). CDs can distort depending on the functional groups of the guest molecules and the nature of the surrounding environment. The results revealed that the actual size range of the guest molecules that all three CDs can complex is greater than expected, compared to the reported cavity sizes. Hydrophilic particles with a diameter of 1.0 - 2.2 Å, hydrophobic particles with 3.2 - 3.6 Å and intermediate particles in the range of 1.0 - 2.4 Å make the complexes with *α*-CD. For *β*-CD these values range from 1.8 - 4.0 Å 3.8 - 5.0 Å and 1.8-3.8 Å and for *γ*-CD, 3.0 - 5.4 Å, 4.8 - 6.0 Å and 2.8-5.4 Å, respectively. However, it should be noted that radial distribution plots of water around the probe particles show that the effective radius of particles is approximately 1.4 - 2.8 Å greater

than the nominal radius. Upon examination of the *β*-CD and drug complexes, there is no rule to include more hydrophobic components of drug molecules within the CD to form inclusion complexes and this agrees with the first part of our study as all hydrophobic, hydrophilic, and intermediate particles can make complexes with CDs with different affinities. Besides, hydrophilic and intermediate probe particles have a tendency to make stable complexes and was confirmed by having highly electronegative atoms of drug molecules in contact with the CD. Figure 2 shows an example complex of *β*-CD a) with continuum probe b) with Ibuprofen.
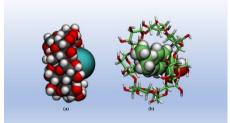

Figure 2: Complex of *β*-CD a) with continuum probe b) with Ibuprofen.

Conclusion
CDs can make complexes with relatively more hydrophilic and intermediate ones even though conventional knowledge assumes complexation occurs with hydrophobic molecules or moeities. Atoms of drug molecules which contact the CD seems to be electronegative ones that may have hydrophilic or intermediate chemical nature. This nature was identified even in the simulation with continuum solid particles where hydrophilic and intermediate particles lie inside the cavity than the hydrophobic particle. The presence of 'anchoring' groups may be important in the stability of these inclusion complexes.

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SYNTHESIS OF *Anonna glabra* MEDIATED SILVER NANOPARTICLES AND THEIR PHOTOCATALYSIS AND TOXICITY ON *Daphnia magna*

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High demand for silver nanoparticles (AgNPs) in different industries has increased its production to extremely prominent levels. Their remarkable properties and unique behavior are the reason behind the intensive use of AgNPs in fields like electronics, appliances, textiles, and medical applications. AgNPs can absorb visible and UV light due to their surface plasmon resonance (SPR) and due to intra-band transition between 4d and 5sp bands (Chouhan , 2019).

Nanoparticle synthesis follows either a top-down or bottom-up approach. In the top-down approach, the bulk material is broken down into nanoparticles. In the bottom-up approach, nanoparticles are stacked up from atoms and molecules. Physical methods of nanoparticle synthesis follow a top-down approach whereas chemical and biological synthetic routes follow a bottom-up approach. Although chemical and physical synthetic methods are sustainable, they are expensive. Hence green synthesis of nanoparticles has emerged as a viable option due to its low cost, low energy demand, and safety (Bharathi *et al.*, 2017)

This study aims at analyzing photocatalytic activity and the toxic potential of the *Anonna glabra* mediated AgNPs against *Daphnia magna*. *A. glabra* which is known as pond apple is a tree native to tropical America and West Africa. It is considered invasive in the Pacific region, Australia, and Sri Lanka.

AgNPs can exert toxic effects in a cellular environment in two ways; by the production of reactive oxygen species and by the release of Ag^+ ions which can denature biomolecules such as proteins. Reactive oxygen species can cause cell death through direct or indirect interaction with intracellular macromolecules, such as DNA, lipids, and proteins (He *et al.,* 2017).

The objectives of the study were,

(I)   To biosynthesize AgNPs using the leaf extract of *Annona glabra* as a green synthetic route

(II)   To analyse their photocatalytic activity and toxicity on *Daphnia magna*.

Methodology

Preparation of plant leaf extract

Annona glabra leaf extract was made by following the method given by Amarasinghe *et. al.* (2020). Briefly, cleaned chopped fresh leaves (20.00 g) were heated with deionized water (100.0 mL) for 1 hour. The supernatant was separated by filtration after cooling the extract to room temperature. The leaf extract solution was refrigerated until further use.

Synthesis of Silver nanoparticles (AgNPs)

Followed the method by Amarasinghe *et al.* (2020). with slight modifications. Dark conditions were used to slow down the synthesis rate in order to avoid the aggregation of synthesized AgNPs. Synthesized AgNPs were further stabilized by stirring in Tween 80 solution (1%, 20.0 mL) for 3 hours. The solution was centrifuged at 600 rpm for 20 min and then the pellet was freeze-dried to obtain dry AgNPs.

AgNPs were characterized using UV-Visible spectrometry, Dynamic Light Scattering technique, electron microscopic analysis, Fourier transform infrared (FTIR) analysis, and X-ray diffraction analysis.

Photocatalytic activity

The photocatalytic activity of AgNPs was tested under an air atmosphere in the presence of sunlight using methylene blue (MB) as described by Vanaja *et al.* (2013). Dye solution (1×10^{-5} M) mixed with AgNPs (25 mg/mL, 2.0 mL) was placed in the dark (30 min) with stirring. Then the solution was exposed to sunlight with slow agitation. At 30 min intervals, for 3 hours, 4 mL aliquots were collected, centrifuged at 6000 rpm (5 min) and the supernatant was analyzed using a UV-Visible spectrophotometer.

Toxicity analysis - Acute Immobilization test with Daphnia magna

An acute toxicity test for *D. magna* was conducted at 27-28 °C according to the OECD guideline 202 (OECD, 2004). Young daphnids aged less than 24 hours (neonates) derived from a healthy stock (i.e. showing no signs of stress such as high mortality, discolored animals, etc.) were used in the toxicity tests. Test solutions of AgNPs with concentrations ranging from (0.01 – 5.00 mg/L) and Ag^+ (0.50 – 2.00 µg/L) were used. Test vessels (sterile plastic containers) were filled with 20 mL of distilled water or test metal solutions. The ratio of air/water volume in the vessels was identical for the test and control groups. Twenty young daphnids, divided into four groups of five animals each, were used at each test concentration and control. The test was conducted using a static system. Each test vessel was checked for immobilized daphnids after 48 hours.

Results and Discussion

SPR band positioned at 417 nm in the UV-Visible spectra confirmed the formation of AgNPs and were polydispersed.

The average size of the NPs according to TEM images varies between 50 – 85 nm with a lesser quantity of NPs with larger sizes between 110 – 195 nm. FTIR spectra confirmed the dual role of biomolecules in the leaf extract as reducing and capping agents of AgNPs. XRD was evidenced for the presence of Ag in AgNPs and average particle size using the Debye-Scherrer formula gave a value of 55.4 nm which is in accordance with the size determined by TEM images.

The absorption peak of methylene blue was observed at 665 nm. Biosynthesised AgNPs show 89.34% of degradation of MB after 3 hours. A higher rate of degradation is observed within 30 minutes.

The percentage immobility of the daphnids was dose-dependent. After 48 h exposure time, 100% immobility of daphnids was observed in 2 and 5 mg/L solutions of Ag^+ and AgNPs. AgNPs and Ag^+ showed a decrease in mobility of *D. magna* with the increased concentrations. Based on tolerance distribution modeling of the concentration and immobility rate relationships, adverse effect concentrations for 50% immobility percentage (EC_{50}) for *D. magna* after 48-hour exposure to AgNPs and Ag^+ were estimated. EC_{50} values obtained as 1.78 ± 0.20 mg/L for AgNPs and for Ag^+ ions it was 1.41 ± 0.20 µg/L. Hence, we concluded that biosynthesized AgNPs exert lesser toxicity to *D. magna* than Ag^+ ions.

Conclusion

The modified method used in this study yielded AgNPs within the nanoscale with optimized conditions of 10% plant extract made by boiling leaves of *A. glabra* at 100 °C, 1 mM

AgNO$_3$ precursor solution and 3 h of incubation. The surface plasmon resonance peak of the formed AgNPs lies around 419 nm with an average size of less than 190 nm. TEM and SEM images confirm the AgNPs are almost spherical in shape and according to these images average size varied between 50 – 85 nm. AgNPs showed photocatalytic activity against methylene blue while more efficient photocatalytic properties could be obtained by stabilizing AgNps with Tween 80. AgNPs exert lesser toxic effects on *Daphnia magna* than silver ions.

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GEOCHEMISTRY AND SEDIMENTOLOGY OF SECONDARY GEM DEPOSITS AT RATNAPURA, SRI LANKA AND GEM EXPLORATION MODELING

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Sri Lanka is famous for valuable gem minerals, which occur as secondary residual, eluvial, and alluvial deposits and are recognized based on their mode of occurrence in geological formations. Gem minerals are liberated from the source rocks by weathering, and then those are entered into the fluvial system either by continuous processes of surface erosion or by episodic events such as landslides and flash floods (Gunatilaka and Almond, 2001). Our study paid special attention to the textural characteristics of gem-bearing sediments in the secondary deposits to distinguish whether they had been released by continuous or episodic surface processes.

Even though enough stratigraphic records are best preserved in sections in the immature profiles of sedimentary gem deposits to assess the sedimentary environment and the paleoclimate, it has not been attempted before. Since it can be assumed that the sediments in the profile of Ratnapura gem beds are in the order of age from Pleistocene to the present, another purpose of this study is to reconstruct the climate history over the Quaternary based on sedimentological studies of the profile of a gem bed.

Currently, there is competition among many gem minors to explore the gems in a limited land area due to the increased population density and the scarcity of gem-prospecting grounds. Therefore, environmental and social impacts due to gem mining and associated activities are rising rapidly. Hence, obtaining an optimum value of resources with minimum disturbances to nature is an essential requirement. Therefore, introducing an effective exploration model to demarcate gem deposits is required for the development of the gem mining industry in the country.

The objectives of the present study were,

(I) Interpretation of the formation of gem-bearing sediments in the Ratnapura area

(II) Understanding the influence of the paleofluvial system on the formation of the gem-bearing profiles

(III) Development of a model for identifying the potential areas for gem deposits

(IV) Interpretation of the paleoclimate over the past

Methodology

Spatial and temporal variation in sedimentological characoristics and interpretation of provenance and paleoclimate

Geology, geomorphology, drainage patterns, and the profiles of the gem pits were studied in the field and using remote sensing data. Approximately, 2 kg soil samples were collected, giving due consideration to the physical variations of the existing layers of selected 12 pristine gem pits without contamination and preserved in polythene bags. In laboratory, samples are subjected to sedimentological analyzes namely the granulometry and the morphometry of grains. The <63 μm sediment size fraction of the samples were selected and pulverized for chemical analysis since it has the highest concentration of clay minerals and most of the trace elements (Wu *et al.*, 2019). The loss on ignition method was used to estimate the organic carbon and inorganic carbon content in each sample. The major and trace elements in <63 μm size fraction of the samples were determined by X-ray fluorescence (XRF) spectrometer. The precision and accuracy of the results were verified with published geochemical data of Highland Complex rocks and stream sediments. From geochemical data, different indices were calculated and diagrams were prepared using major oxides. ARC GIS 10.4.1 software was used as the Geographic Information System (GIS) platform for analysing spatial data.

Drainage morphometry, morphology and their influence on the formation of gem-bearing profiles

Based on the Advanced Space born Thermal Emission and Reflection Radiometer (ASTER) 30m Global Digital Elevation Model (GDEM), drainage network delineation and quantitative morphometric analysis was carried out for the demarcated 8 sub-watersheds of Kalu Ganga basin in Arc GIS environment. The slope map of the study basin was obtained using the same ASTER GDEM, and reclassified to 10 intervals and smoothed. The surface roughness index of the study basin was calculated, and then the surface roughness map was generated and stretched to differentiate smooth and rough surfaces.

Develop a GIS model to identify the potential areas for secondary gem deposits

The overall methodology of the study can be framed into five steps: (i) development of criterion (ii) acquisition of base data (iii) preparation of factor maps (iv) exploration modeling (v) accuracy assessment. The developed criterion lithology, geological structures, landforms, and river basins were identified as possible factors for the identification of gem potential areas and then the exploration modelling was done using the factor maps for the the Kalu Ganga. An analytical Hierarchy Process was used to calculate the weight of each factor. An error matrix method was applied for the accuracy assessment of the model.

Results and Discussion

Spatial and temporal variation in sedimentological charactoristics and interpretation of provenance and paleoclimate

The sorting of Denawaka and Ratturugala gem-bearing sediments shows that they have been less transported from the source, while Thiruwanaketiya gem-bearing sediments may be from a more distant source or may have been formed through more than one cycle of sedimentation/re-worked sediment by the fluvial system. Based on the mean size and the Skewness, it can be suggested that the gem-bearing sediments in Thiruwanaketiya have been deposited in a sheltered, low energy environment, while those in Ratturugala and Denawaka have been deposited in steadily decreasing energy conditions. Some major and trace elements of the studied gem-bearing sediments show an apparent positive correlation with Al_2O_3, confirming clear hydraulic fractionation. The geochemical proxies, namely SiO_2/Al_2O_3 ratio, Chemical Index of Alteration (CIA), Index of Compositional Variation (ICV), Plagioclase Index of Alteration (PIA), and Weathering Index for Sediment (WIS), show higher chemical weathering in their source areas and low degrees of maturity of gem-gearing sediments. The A-CN-K plot suggests that the studied gem-bearing sediments are from albitic-rich sources with less K mobility.

Further, the gem-bearing consolidated ferruginous hard layer in Ratturugala which plots much closer to smectite in the A-CN-K diagram, denotes contrasting seasons and a pronounced dry season. The bivariate plot SiO_2 vs. $(Al_2O_3+K_2O+Na_2O)$ of the studied gem-bearing sediments displays that those have been subjected to sub-oxic semi-arid to arid climatic conditions. The Mn* values of the studied samples suggest that they may have been deposited in reduced environmental conditions. The landslides during high precipitation may have probably deposited gem-bearing sediments in Denawaka. The bottom-most gem-bearing sediments lie on the decomposed bedrock, and this indicates that a river course did not exist prior to the deposition of gem-bearing sediments and/or the drainage system may have become locked into the geological structures a long time ago. The hydrologic process in hillslopes and zero-order basins has controlled the stream-flow generation and stream chemistry of Kalu Ganga. The organic matter rich layer present above the gem-bearing layer in Denawaka and Pathakada stratigraphic profiles may represent a lake, swamp, or marsh-type flood basin environment in the past.

Drainage morphometry, morphology and their influence on the formation of gem-bearing profiles
The river basin's morphometric parameters indicate that the Kalu Ganga Basin has an arid climate and a high tendency to have frequent landslides and floods during the rainy season. This implies that landslides and floods may have played a major role in the formation of sedimentary gem deposits in the Kalu Ganga Basin, Ratnapura. The morphological analysis indicates that the study area consists of six major landforms, namely alluviums, toe slopes, open slopes, middle slopes, upper slopes, and hills/ridges. The generated surface roughness map is useful for predicting the potential deposition of gem-bearing alluvial sediments. River cross-sections confirmed that Kalu Ganga has been subjected to lateral migration, cutoff development, avulsion, and incision due to allogenic factors. This implies that paleoclimatic incidents such as high rainfall, landslides, and base level fluctuations have affected the course of the paleofluvial system of Kalu Ganga and its tributaries. Accordingly, landslides and floods have played a major role in the formation of sedimentary gem deposits in the Kalu Ganga Basin, Ratnapura.

Develop a GIS model to identify the potential areas for secondary gem deposits
There is a closer relationship between lithology, geological structures, geomorphology, stream network, and secondary gem deposits in Ratnapura gem field. The predictive APH and GIS based model is unequivocally confirmed this relationship. The gem potential map broadly identifies the target areas for evaluation through detailed exploration. The model identifies four gem potential classes, namely, High Potential, Moderate Potential, Low Potential, and Least Potential. The cross-validation of the GIS model by the error matrix method showed a 72% correctness for the High Potential + Moderate Potential areas. The sites classified as either High Potential or Moderate Potential can be considered suitable for economic gem mining.

Conclusion
Secondary gem deposits in the area have been subjected to a series of geological processes, beginning with regolith formation, followed by mass wasting processes caused by channelized landslides, and finally fluvial activities. Gem-bearing sediments were hydraulically fractionated under semi-arid to arid climatic conditions. Some gem-bearing sediments are characterized by immature sediments. Several gem-bearing sediments may have formed from distant sources, and some have been subjected to more than one cycle of sedimentation processes. The wavelet analysis of sedimentological proxies (textural and geochemical) of the studied stratigraphic profile indicates that this region has been in semi-arid to arid, higher weathering, and reduced environmental conditions, with a variable precipitation trend ranging from extremely high to medium throughout the represented Quaternary period. The highly cemented consolidated gem-bearing ferruginous layers in the studied stratigraphic profiles indicate that they have been subjected to sub-oxic conditions. The morphometric analysis enabled to predict gem potential alluvial deposits with higher accuracy and at a lower cost. The developed gem potential model is a cost-effective method for evaluating possible gem potential areas on a regional scale, despite their limitations.

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BIOSORPTION OF HEAVY METAL IONS ON *Artocarpus nobilis* FRUIT PEEL AND ITS APPLICATIONS FOR TREATMENT OF INDUSTRIAL EFFLUENTS

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Heavy metals are considered as essential metals which affect socio-economic and cultural aspects of the human community. In historical society, heavy metals have been used in many applications without proper understanding of their nature and influence on the environment. Consequently, wastewater containing heavy metals and their compounds having a broad range of concentrations was released to the environment. Risks of heavy metals in the environment were later found with significant issues, such as Itai-itai disease (Inaba *et al*., 2005), (Horiguchi *et al*., 1994), and Minamata disease (Harada, 1995) in Japan. Toxicity of heavy metals and their ability of bio-magnification through the food chain directly affect aquatic fauna and flora. Heavy usage of heavy metals was reported to contribute to water pollution in developing countries (Shroff and Vaidya, 2011).Therefore, mitigation techniques should be introduced considering the physical and chemical properties of such metals that are discharged to the environment. Biosorption is a new trend that is successfully implemented for removal of heavy metals present in wastewater owing to many advantages, such as cost effectiveness, ability of saving energy, ease of applying in industrial purposes, need of small amounts of adsorbent/biosorbent, ease of regeneration and minimization of sludge formation. Therefore, the fruit peel of *Artocarpus nobilis*, an endemic plant to Sri Lanka, was used as an effective biosorbent in this research. Artocarpus species are rich in phenolic compounds demonstrating high affinity toward metals (Jayasinghe *et al*., 2006). Characteristic features of the interfacial region of the biosorbent - heavy metal ions play an important role in biosorption. Complications however arise due to the fact that organic functional groups present in the biosorbent and different sizes of pores (macropores, mesopores and micropores) of the surface would not be dispersed homogeneously. Therefore, the rate of biosorption of heavy metal ions would be fluctuated depending on the position of the surface of the biosorbent. This problem would be overcome to some extent by modification

of the biosorbent as the modified surface would act as a new surface possessing properties of the modifier. Consequently, organic functional groups and the surface area could be altered for favorable biosorption. The objectives of the research study were,

(I) Investigation of interaction between synthetic heavy metal solutions, and the peel of *Artocarpus nobilis* fruit in natural and modified forms under static and dynamic conditions

(II) Application of the peel of *Artocarpus nobilis* fruit for successful removal of heavy metals from industrial effluents through the development of a prototype heavy metal treatment plant

Methodology

Matured and ripen fruits of *Artocarpus nobilis* were collected from different locations of low and central highlands of the wet zone of Sri Lanka. Fruits collected were washed thoroughly with tap water for removing dust, solid and sand particles. Outer peel of fruits was removed and allowed to air dry under ambient condition. Thereafter, dried peels were thoroughly mixed, sieved using 1.00 mm and 0.71 mm sieves, and the fraction of biosorbent of the diameter in the range of $0.71 < d < 1.00$ mm was used for all static and dynamic experiments. Raw peels were washed with tap water followed by distilled water. Washed peels were then air dried and used for static/dynamic experiments and for the prototype heavy metal treatment system designed in this research. Another part of raw peels was modified using NaOH, and thereafter, the wet peels were air dried and kept for both static and dynamic experiments. Accordingly, three types of peels were prepared in this research; raw peels, peels washed with distilled water and NaOH- treated peels. Each type of peels was separately treated with standard solutions of Cd(II), Cr(III), Cu(II), Ni(II), Pb(II) and Zn(II). Spectro-electronic Thermo M series atomic absorption spectrophotometer (AAS)was used to determine the total concentration of each metal ion in solutions. Finally, synthetic Ni(II) solution was used for the operation of heavy metal treatment plant.

Results and Discussion

In order to understand the adsorption behavior of the biosorbent, it is important to identify its characteristics, such as active functional groups, specific surface area and surface charge. The surface charge of a biosorbent is dependent of the pH of the medium, and it can be positive, negative or zero due to the activities of potential-determining ions and electrolyte concentrations. The point of zero charge (PZC) of the biosorbent was estimated in the pH range of 3.5 – 4.5. The biosorbent surface is negative when the pH of the solution > $pH_{(PZC)}$, which is a good feature for the metal biosorption process. X-ray fluorescence analysis is indicative of the presence of K, Ge, Cu, Fe and Ca as major metals present in the biosorbent. Scanning electron microscopic images show that the surface of the raw biosorbent is rough which helps to trap metal ions. When compared with the raw biosorbent, the surface of the Ni(II)-treated biosorbent has become smoothened and covered with a layer of adsorbate, probably Ni(II) attached on the surface of the biosorbent. These observations indicate the possibility for Ni(II) ions to be trapped and adsorbed on the peel surface. Fourier transform infrared spectroscopy provides evidence for the presence of many organic functional groups, including –COOH, –COOR, –CHO and –OH. These organic functional groups have the affinity for heavy metal ions which becomes stronger in basic medium due to the formation of negatively charged ions.

Biosorption depends on several factors, including shaking time, settling time, initial solution pH, and pre-treatment conditions, such as heating time / temperature of biosorbent and treatment of the biosorbent with the modifying agents. Optimization experiments were thus conducted with separate solutions of 10.0 mg L^{-1} of Cd(II), Cr(III), Cu(II), Ni(II), Pb(II) and Zn(II). High removal percentages of 88%, 70%, 80%, 74% and 92% were recorded for Cd(II), Zn(II), Cr(III), Cu(II) and Pb(II), respectively under optimum conditions. Further, the biosorption process is pH dependent, and thus the removal percentages and the maximum adsorption capacities of metal ions were changed depending on the pH of the background environment. Moreover, the hydrated radius of Ni(II) ion at soluble media is very high compared to that of other metal ions.

Therefore, the lowest removal percentage of 50% was recorded for Ni(II). The adsorption capacities (q_{max}) investigated using the Langmuir adsorption isotherm model leads to the highest value of 26,316 mg kg^{-1} for Pb(II) and the lowest value of 9,804 mg kg^{-1} for Zn(II). Nevertheless, the q_{max} of Ni(II) was comparatively high despite the lower removal percentage. Therefore, the Ni(II) was selected for operation of the prototype heavy metal treatment plant in the final stage of this research. Solution analysis after achieving adsorption equilibrium with distilled water washed peels (DWP) and NaOH-treated peels (STP) indicate that removal percentages and q_{max} are significantly affected, which is very important for the application of the biosorbent in commercial basis for heavy metal treatment. The specific surface area (S_s) of the peel has been increased dramatically under the NaOH treatment due to the destruction of structural materials, such as Lignin present in the surface, and the S_s values of DWP and the peels treated with 0.1 mol l^{-1} NaOH were calculated to be 382 and 919 m^2 g^{-1}, respectively.

The synthetic Ni(II) solutions were used for treatment in the prototype treatment plant considering the effluent quality of industries in Sri Lanka. Continuous flow stirred tank reactor and the plug flow reactor were used for designing the prototype heavy metal treatment system. This arrangement reduces the effluent concentration in two stages. The operation of the treatment plant was continued for 38 days until the initial and final concentrations of Ni(II) were equal, and the deterioration or changing appearance of the biosorbent and even evolving anaerobic gases were not observed during the entire operation time period of the treatment plant. Moreover, the biochemical oxygen demand and the chemical oxygen demand were remained at low levels up to the final day of the operation, further demonstrating the effectiveness of the treatment plant. Although the pH values were slightly fluctuated around the average of 6.63, all parameters tested were complied with the tolerance limits for the discharge of industrial effluents into inland surface water under the National Environmental Act of Sri Lanka.

Conclusion

Development of environmentally friendly techniques for removal of heavy metals in industrial effluent is a big challenge in current situation. This research successfully focused on this challenge using the peels of *Artocarpus nobilis* fruit as a biosorbent for removal of heavy metals.

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HIGH-STRENGTH WASTEWATER TREATMENT USING COMBINED BIOLOGICAL AND PHYSICOCHEMICAL METHODS

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High strength wastewater (HSWW) such as landfill leachate contains elevated amount of organic matter and comparatively high amount of nutrients is common. It might further characterized by heavy metals, organic acids, bases, oil and grease, microorganisms and many other recalcitrant organics according to the source materials. Therefore, HSWW is a broad topic in terms of constituents and somewhat difficult to treat in order to meet the discharge limits. With the stringent effluent discharge limits, engineers need to come up with better treatment processes day by day. On the other hand, with the exponential growth of large urban centers, the space for the installation of treatment plants to treat wastewater generated by households and industries is not enough. Therefore, the construction of treatment plants that enable compact installations, occupying the least possible space is becoming important.

Moving bed biofilm reactor (MBBR) is a fast-growing technology for wastewater treatment (Kawan *et al.,* 2016). It is an advanced high-rate wastewater treatment technology with high treatment efficiency, small footprint, low capital, operational, maintenance, and replacement cost; single reliable, and robust operation procedure (Barwal & Chaudhary, 2014). Much of the development work on the MBBR process with plastic biofilm carriers was done with the aim to provide not only organic removal but also compact biological nitrogen removal to reduce point source discharges. The new technologies such as membrane filtration are proven to be effective, however, the cost associated with such new technologies may not be applicable to developing countries. Maintenance difficulties due to a lack of skilled personnel are further problems. Therefore, investigating an alternate cost-effective treatment system that has the ability to treat mixed high-strength wastewaters having low biodegradability, along with identifying the maximum tolerance level of the system, would be an important investigation.

The main objective of this study was to identify effective high-strength wastewater treatment options, especially for developing countries in the tropical belt. Accordingly, sub-objectives were,

(I) To identify the characteristics of high-strength wastewater

(II) To investigate the efficiency of a combined conventional system in treating HSWW

(III) To investigate the efficiency of MBBR technology in treating HSWW

Materials and Methods

Initially, the characterization of landfill leachate collected from selected local municipal solid waste (MSW) landfills was performed in order to correctly understand the characteristics of local landfill leachate conditions. Raw leachates were collected from four landfill sites; Karadiyana, Gohagoda, Dompe, and Matale, and were analyzed for quality. General water quality parameters pH, EC, temperature, organic matter contents in the forms of BOD_5, COD and TOC, TN, and typical anions such as SO_4^{2-}, NH_4^+, NO_2^-, NO_3^- and PO_4^{3-} were measured in three different months. Then, the MBBR system and combined stabilization pond system were operated to investigate the ability to treat HSWW having low biodegradability, along with identifying the maximum tolerance level of the systems. The pilot scale pond system: anaerobic pond, facultative pond, aerobic pond, and constructed wetland operated in tropical conditions for leachate treatment. The lab-scale integrated MBBR treatment system of operated in steady and continuous flow conditions. Anaerobic and aerobic MBBR was combined with a constructed wetland to investigate the effectiveness of a combined system in treating landfill leachate blended with dairy wastewater and septage. Blending was done to have a better BOD_5/COD ratio and organic loading rates (OLR). Finally, the advantages and disadvantages of the systems were compared in terms of cost-effectiveness, and treatment efficiency.

Results and Discussion

Landfill leachate quality and quantity can vary from one location to another depending upon factors such as the type of MSW dumped, site hydrogeology, waste moisture level, landfill design, operational procedure, age, relative biodegradability of waste, annual rainfall, temperature, etc. (Wang *et al.,* 2003).

Overall, the leachate from tested sites was characterized by high ammonia concentrations and low nitrate and nitrite concentrations. The lowest average nitrate, nitrite, and also ammonia values in leachate also were reported from Dompe landfill which is the youngest. The BOD_5/COD ratio of Karadiyana and Gohagoda leachates which are older landfills ranged between 0.01-0.05. The higher COD values at Karadiyana may be due to the nature of the waste disposals at this facility compared to others. The pH values of leachate were around 8, which is a comparatively higher value compared to standard norms implying that the leachates are not young leachate. Biological degradation converting organic nitrogen into ammonium with the age of a landfill is well documented as the main cause for higher pH in leachates.

In summary, the pond system performance improved with the maturation process, and 93 to 99% overall COD, ammonia, and BOD_5 removal efficiencies were obtained with ORLs ranging from 0.13 - 0.30 kg COD m^{-3} day^{-1}. Raw leachate characteristics too may have affected the higher efficiencies. Moreover, the higher efficiencies indicate that the hydraulic retention times (HRTs) and OLRs used here were ideal for each stabilization pond and wetland, under tested conditions. Combining wetland with the stabilization pond system leads to improved results and it is a viable alternative for the polishing stage in treating landfill leachate in tropical environments.

The start-up stage results indicated that the combined MBBR system takes a comparatively shorter period of time around 20 to 30 days to reach the stabilized stage. With the increase in OLR, better removal rates were observed. This implies that the capacity of the system is increased with the organic loading in the system. Such results can be attributed with the increase in biomass in the reactors simultaneously to the increase in substrate concentration in the MBBRs. As the feed COD level increased, the effluent COD decreased, increasing the efficiency. With the sudden change in OLR, the overall MBBR COD removal efficiency fluctuated between 88% and 86% implying that the system has the ability to withstand high OLRs at reduced HRTs too. The anaerobic MBBR was responsible for the majority of ammonium-N removal, possibly due to the Anammox process, dilution effect, and agitation. The ammonia stripping using a high mixing rate can eliminate NH_3-N and the process is termed "agitation" because it depends on the agitation rate or rotating speed

subjected to the wastewater surface. Since the conventional nitrogen removal systems require a large amount of energy for high-rate aeration for biological nitrification, the anaerobic MBBR can consider as a suitable alternative cost-effective process that requires comparatively less energy. Furthermore, the anaerobic ammonium oxidation was found to be most suitable for higher temperature operation conditions around 28-35 °C in which maximum rates are obtained, it is applicable in facilities in high-temperature zones rather than low-temperature areas, where additional costs associated with temperature-maintained at a higher level than room temperature.

Additionally, a microbiological investigation was also conducted along with the MBBR study to identify the available microbe community in different reactors.

Conclusion

According to the study, the combined anaerobic-aerobic MBBR system achieved over 75% efficiency at 7.6 kg m^{-3} day^{-1} organic loading rate in the leachate blended dairy wastewater fed system, and over 77% efficiency at 4.6 kg m^{-3} day^{-1} organic loading rate in the leachate blended septage fed system. Although higher organic loading rates showed a fall in removal efficiency, a net increase in total organic and nutrient removal rates was observed. Furthermore, the septage study showed that the blended wastewater characteristics can play a vital role in overall efficiency. In comparison with MBBR, the pond system achieved lower organic removal. Though it can only be operated in comparatively low organic loading rates, the pond system showed well-absorbed load fluctuations, maintaining a fairly constant reliable overall COD removal under varying influent conditions. Microbiological investigation claims that microbes having the ability to remove phosphate from wastewater are present in the MBBR reactors tested in this study.

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EFFECT OF SOIL AMENDMENT WITH SOLUBLE SILICON ON HOST RESISTANCE, PRE- AND POSTHARVEST DISEASES AND QUALITY OF BITTER GOURD (*Momordica Charantia* L.)

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Bitter gourd/bitter melon (*Momordica charantia* L.), also known as the 'natures silent healer' is one of the most popular cucurbit vegetables in Southeast Asia (Guptha *et al*., 2011). However, bitter gourd plants are highly susceptible to many fungal diseases at different growth stages leading to a significant loss of yield. The warty nature of the bitter gourd fruit surface makes them more susceptible to damage during postharvest handling and thereby to subsequent colonization of fungi. Although, the use of synthetic fungicides is the primary means for controlling preharvest and postharvest diseases in bitter gourd, adverse effects associated with the use of synthetic fungicides have generated an interest in developing safer, eco-friendly alternatives for the control of preharvest and postharvest diseases of crops (Nigro *et al*., 2006).

Silicon (Si) being the second most abundant element in the soil, covering approximately 28% of the earth crust has shown a number of beneficial effects on plant growth, mineral nutrition and especially on stress tolerance to biotic and abiotic stress factors in a wide range of crops (Epstein, 1994). Si has been exploited for its beneficial effects in terms of disease control in many plants including rice, wheat, sugarcane, zucchini, peach and cucurbits (Liang *et al*., 2015).

The mechanisms responsible for the protection of plants from diseases by Si are not well elucidated yet. However, several studies have shown that lower disease severity in Si-treated plants was in line with structural reinforcement of plant tissues which acts as a physical barrier that interfere with pathogen penetration (Rodrigues and Datnoff, 2015), inhibiting pathogen colonization through stimulating systemic acquired resistance, production of antimicrobial compounds and increasing plant resistance by activating multiple signaling pathways and defense-related gene expression (Rodrigues and Datnoff, 2015). In this context, better understanding of protective effects of Si will open up a new avenue for use of silicon, as

a potential alternative source to fungicides in bitter gourd.

Objectives

(I)   To determine the effectiveness of soil-amendment with Si for controlling preharvest foliar fungal diseases and postharvest fungal diseases of bitter gourd.

(II)   To investigate the nature of possible protective effects against fungal pathogens with special emphasis on structural and chemical changes in bitter gourd leaves and fruits.

(III)   To ascertain the effectiveness of soil amendment with rice husk (a natural source rich in Si) in controlling fungal diseases as a low-cost, environment-friendly method to be adopted by local bitter gourd growers.

(IV)   To ascertain the impact of applied rice husk ash on nutritional and keeping quality of bitter gourd

Methodology
Effect of silicon application on plant growth and pre-harvest fungal diseases of bitter gourd leaves

The experiments were conducted as pot trials using sand: top soil: compost as the growth medium at 2:1:1 ratio. Three seeds of bitter gourd cultivar Mathale Green were planted per pot and thinning to one seed was done at four leaf stage. Potassium silicate (20% SiO_2; 80% K_2O; Daejung, Korea) in three concentrations, 100 mg Si kg^{-1} soil (100 ppm), 200 mg Si kg^{-1} soil (200 ppm) and 300 mg Si kg^{-1} soil (300 ppm) were added to the growing medium at the four leaf stage and continued up to 28 days at seven-day intervals. Media without silicon but added with potassium fertilizer in appropriate amounts to compensate the effect of added potassium (in the form of K_2SiO_3) were used as the control. Each treatment consisted with fifteen pots and the experiment was repeated twice.

Potting media with added fertilizer were analyzed for plant-available Si and pH before amendment with Si. Silicon accumulation in leaves was analyzed from 35 days after the first Si application and continued up to 77 days at seven-day intervals using the procedure of autoclave-induced digestion (Elliot and Snyder, 1991). Thirty five days after the first Si application, plants were inoculated with

Erysiphe sp., the powdery mildew pathogen, or *Pseudoperonospora* sp., downy mildew pathogen (Remus-Borel *et al.*, 2005), the most common foliar fungal pathogens during the experimental period and observed daily for disease progress. Area under disease progress curve (AUDPC) for each treatment was calculated by the midpoint rule method (Campbell and Madden, 1990) and the plant growth parameters also were tested. The best Si application rate was selected based on the results.

Defense responses enhanced by soluble silicon in bitter gourd-powdery mildew pathosystem
Plants were grown on potting mixture as described above and 35 days after the first Si application, plants were inoculated with *Erysiphe* sp. Three days after pathogen inoculation, plant leaves excised from each treatment were extracted and used for identification of antifungal compounds, phenolic constituents, defense-related enzyme activities; peroxidase, polyphenol oxidase and pathogenesis-related proteins; chitinase and β-1,3-glucanase. Transverse sections of leaves were observed under light microscope and the cuticle–epidermal layer thickness was measured. Scanning electron microscope coupled with EDX detector (Energy dispersive x- ray spectroscopy) was used to observe and quantify Si deposition on leaves (Pathan *et al.,* 2010).

Effect of root application of soluble silicon on postharvest quality of bitter gourd
Bitter gourd plants were grown on the potting mixture as described above and the fruits were harvested at the maturity. Postharvest quality of bitter gourd was tested with special reference to disease development by *Fusarium* sp., the major pathogen at the postharvest stage.

Effect of soil application of rice husk as a natural silicon source on some preharvest and postharvest quality of bitter gourd
Field experiments were conducted as above and the potential of Rice husk in three different forms; ground raw husk (GRH), partially burned rice husk (PBRH) and rice husk ash (RHA), as a natural Si source on pre-harvest and postharvest quality was evaluated by repeating all the analyses described above. Further, impact of Si on major nutrients, N, P, K, crude protein, crude fiber, crude fat and ash content were also tested.

Results and Discussion

The soluble Si level in the potting mix before applying the Si treatments was very low (2.87 mg kg^{-1} Si, 0.47 mM) and pH was nearly neutral (7.2). The soil used for the current study was suitable for Si application experiments since Si is found in the soil solution as solubilized monosilicic acid [$Si(OH)_4$] at a pH < 9 (Raven, 1983).

Further, effect of Si concentration, 200 mg Si kg^{-1} soil on controlling powdery mildew and downy mildew as well as on increasing number of leaves, flowers and fruits was highly significant (p < 0.05) thus it was identified as the optimum Si level to be applied in the next experiments.

Si accumulation on epidermal cells, stomata, intercellular spaces and on glandular trichomes of the leaf was observed and cuticle - epidermal layer thickness in Si treated leaves (19.3 μm) was significantly higher (p < 0.05) than that of control plants (12.1 μm). Si also induced biochemical defense responses significantly in leaves by elevating the activities of enzymes; peroxidase (POD), polyphenol oxidase (PPO) and pathogenesis-related proteins; chitinase and β-1,3-glucanase. Cladosporium bioassay coupled with Thin Layer Chromatography has revealed the presence of both preformed and induced antifungal compounds and some of them were identified as phenolics.

Soil amendments with Si improved the growth parameters and postharvest quality of bitter gourd and extended the shelf life by 2 days compared to the control. Weight loss of fruits harvested from Si treated plants was significantly lower than that of control and it delayed yellowing while reducing Fusarium rot development in fruits by enhancing the activity of antifungal compounds. A significant increase in POD and PPO activities was observed in Si treated fruits regardless of inoculation with the pathogen, although the observed increase of chitinase and β-1,3-glucanase in bitter gourd fruits was insignificant.

Application of rice husk ash exhibited similar effects on both leaves and fruit of bitter gourd. Rice husk ash improved growth and yield characters and enhanced resistance to foliar fungal pathogens in bitter gourd. The enhanced disease resistance appears to be positively associated with the higher accumulation of silicon and Si-induced phenolic content and increased activity of PPO and POD enzymes in leaves. It also improves some nutritional factors including fruit N and P content, crude protein, fiber and fat content while increasing antioxidant properties. At the consuming stage, there was no significant difference in Si content of both Si treated and control fruits.

Conclusions

The present work identified the efficacy of soluble Si (optimum level 200 mg Si kg^{-1} soil) on controlling preharvest foliar diseases; powdery mildew and downy mildew and postharvest disease, Fusarium rot in bitter gourd. Disease reduction was achieved through induced chemical defense responses as well as by reinforcement of structural defenses. Rice husk ash can be applied as a natural Si source and all the beneficial effects obtained by applying potassium silicate can be achieved through rice husk ash too. The findings of the research introduce a novel technology to manage preharvest and postharvest diseases in bitter gourd cultivar Mathale Green that can be adopted by farmers in Sri Lanka.

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SYSTEMATICS OF GENERA *Dendrobium* Swartz AND *Bulbophyllum* Thouars (FAMILY: ORCHIDACEAE) IN SRI LANKA

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Orchidaceae is one of the most species-rich plant families in angiosperms and the most recent figure is 26,567 species and 736 genera (Dressler, 1993 and Chase *et al.*, 2015). They can be found in every biome type except true deserts and polar regions (Jayaweera 1981). Among those orchids, *Dendrobium* and *Bulbophyllum* species are broadly categorized into horticultural, agricultural, medicinal or dual purpose species depending on their utility. The literature on Sri Lankan species of genera *Dendrobium* and *Bulbophyllum* has not revealed their commercial applications. Further, any comprehensive taxonomic treatments for these genera have not been undertaken in the past four decades apart from the treatment by Jayaweera (1981). According to the IUCN Redlist (2012), many species of the genera *Dendrobium* and *Bulbophyllm* are in high risk in nature. In the genus *Dendobium*, *D. maccrthiae*, *D. heterocarpum* and *D. salaccencse* are endangered species while *D. panduratum* and *D. nutantiflorum* are near threatened species. The *D. diodon* is categorized as vulnerable. *D. aphyllum* is the only species under least concerned category in the genus *Dendrobium.* Considering the records of genus *Bulbophyllum* in the IUCN Redlist (2012), three species; *B. crassifolium*, *B. maskeliyense* and *B. purpureum* are endangered and six species; *B. elegans*, *B. macraei*, *B. petiolare*, *B. thwaitesii*, *B.trimenii* and *B. wightii* are vulnerable. *B. elliae* is categorized as a near threatened species while *B. tricarinatum* is categorized as a critically endangered species. *B. jayaweerae* is a data deficient species. Therefore, habitat destruction has threatened the survival of many more species throughout the range of the genera *Dendrobium* and *Bulbophyllum.* Considering the above mentioned facts, present study was aimed to revise the genera *Dendrobium* and *Bulbophyllum* in Sri Lanka using morphometric and ITS sequencing data and to find the host tree specificity of these genera as that would undoubtedly create the interest of not only botanists, but also the commercial orchid growers, ecologists and conservationists.

The objectives of the present study were,
(I) To carry out a detailed morphometric study using both quantitative and qualitative characters of genera *Dendrobium* and *Bulbophyllum* in Sri Lanka and to prepare a pictorial guide and a multi-access key.
(II) To conduct multiple sequence analysis of internal transcribed spacer (ITS) region of some species of *Dendrobium* and *Bulbophyllum* in Sri Lanka.
(III) To perform a multivariate approach to infer phenetic relationships of species of genera *Dendrobium* and *Bulbophyllum* based on the morphomertry and phylogenetic relationships of some species of two genera based on ITS sequence analysis.
(IV) To assess the host plant preference of genera *Dendrobium* and *Bulbophyllum* in Sri Lanka.

Methodology
Plant material
Wet forests in Sri Lanka were explored by fifty field visits to collect species of the genera *Dendrobium* and *Bulbophyllum* in several field trips from December 2009 to July 2012. Six species of genus *Dendrobium* and four species of genus *Bulbophyllum* which were diagnosed by flower were collected by the field survey. Therefore, present study focused on *D. aphyllum*, *D. crumenatum, D. diodon, D. heterocarpum, D. nutantiflorum* and *D. panduratum, B. elliae, B. petiolare, B. trimenii* and *B. wightii.* Collected specimens were identified compared to authenticated specimens at the National herbarium, Peradeniya, Sri Lanka and floral drawings and descriptions in Flora of Ceylon by Jayaweera (1981). The nomenclatural priority was given to Fernando and Ormerod (2008).

Morphometrics
Thirty two morphological characters; seven vegetative and twenty five reproductive/floral characters were recorded, of which six were qualitative and twenty six were quantitative. Floral parts of dissected flowers and cross sections at mid-region of the leaf were drawn with the aid of a stereomicroscope equipped with a camera lucida (Olympus SZX 7) and measurements were then made from the drawings at the point of maximum dimension. The data matrix for the multivariate analysis was completed by the studied herbarium

specimens and the descriptions by Jayaweera (1981). *D. bambusaefolium* was not encountered for the analyses because lack of data from herbarium specimens and descriptions by Jayaweera (1981). All the data were transformed into binary state data and cluster analysis of the species was carried out by PAST 3.1 software package using the Euclidean distance treating with the Single Link Resolution and UPGMA as the sorting strategy. Principal Component Analysis (PCA) on a correlation matrix was used as an objective method to show the variation among the species and among the selected variables using CANOCO for Windows 4.5 software package. The number of axes to interpret was determined by comparing eigenvalues. All the studied characters of the taxa were gathered on a multi-access key using the DELTA software package. The key was constructed using photographs, illustrations and both qualitative and quantitative characters of floral and vegetative parts.

ITS sequence based phylogenetic relationships
Plant genomic DNA was extracted at the laboratory, Department of Molecular Biology and Biotechnology, University of Peradeniya, Sri Lanka following the modified CTAB protocol by Russell *et al*. (2009). The DNA from all species was subjected to polymerase chain reaction (PCR). The internal transcribed spacer (ITS) region was amplified as described by Hidayat *et al.* (2007) using the primer sets of 17SE and 26SE. Sequencing was carried out by ABI 3500 genetic analyser (Applied Biosystems®) using Sanger method. BLAST search was performed for all the obtained ITS sequences and to verify the sequences of other recorded species in the website of the NCBI. Phylogenetic analysis was performed to infer the evolutionary relationships of the species compared to mainland India and associated islands in the Indian Ocean using available ITS sequences in the NCBI database.

Host plant specificity
While orchid samples were collected randomly within about 500 m^2 area in the field, data of host plants were recorded; tree height, girth at breast height (GBH), characteristics of tree barks (bark colour, nature of the moss cover and bark texture) and the abundance of orchid species on the host tree (whether single plant, few plants or a plant population present). Further, herbarium specimens were prepared.

Identified herbarium specimens were authenticated against the specimens in the National herbarium, Peradeniya, Sri Lanka.

Results and Discussion
Clustering pattern of *D. panduratum* & *D. diodon* and *B. purpureum* & *B. tricarinatum* has indicated their close relatedness with respect to the similarity in floral morphology. The relatedness is more distinct in both quantitative and qualitative floral morphological characters than to their vegetative morphology. PCA has suggested that shape of lateral sepals and petals, number of veins in sepals, apex angle of petals and lateral sepals, width of flower, lateral sepals, petals and column, length of lip and column and shape of the leaf apex of *Dendrobium* spp. and pseudobulb diameter, distance and floral characters; length of sepals, column and lip, width of flower, lateral sepals and lip, number of veins in sepals, dorsal sepal shape and apex angle and shape of bract of *Bulbophyllum* spp. as most influential characters in delimiting the taxa and deriving interspecific variations. Further, *Eria bicolor* (outgroup) has aligned with *Pinalia spicata* in the multiple sequence alignment of ITS sequence suggesting the agreement of consideration of the placement of *E. bicolor* under genus *Pinalia* in the recent molecular level approach. The phylogenetic trees derived based on ITS sequence analysis of species of *Dendrobium* and *Bulbophyllum* using *Eria* sp as the outgroup are in agreement with suitability of *Eria* as an out group taxa. With respect to the host specificity, *Dendrobium* spp. have shown affinity to the genus *Glochidion* (family Euphorbiacea) whereas *Bulbophyllum* spp. have the preference to genus *Syzygium* (family Myrtaceae). Derived host relations would facilitate future conservation programmes of potential host plants thereby enable the establishment of rich wild orchid flora in the respective forests.

Conclusions
Morphometrics of present study suggests the importance of analyzing both qualitative and quantitative characters for the inferring relationships of taxa in genera *Dendrobium* and *Bulbophyllum.* Further shape of the lateral sepals and petals, number of veins in sepals, apex angle in petals and lateral sepals, width of flower, lateral sepals, petals and column, length of lip and column and shape of the leaf apex are

the most significant morphological characters for deriving the intraspecific variation of genus *Dendrobium*. Also pseudobulb diameter and distance, and floral characters; length of sepals, column and lip, width of flower, lateral sepals and lip, number of veins in sepals, dorsal sepal shape and apex angle and bract shape are the most significant morphological characters to derive intraspecific variation of genus *Bulbophyllum*.

ITS sequence based phylogeny of the study confirms the fact nrITS sequences are provided sufficient evidence in inferring phylogenetic relationships of some species of *Dendrobium* and *Bulbophyllum* in Sri Lanka as in Wonnapinij and Sriboonlert (2015). In the phylogenetic relationships inferences of *Dendrobium* species and *Bulbophyllum* species, genus *Eria* can be selected. Further multiple sequence alignment results of ITS sequence have provided valuable information for the revision of the placement of *E. bicolor* and in agreement with the suggestion of placing under genus *Pinalia* by Fernando and Ormerod (2008). But any evolutionary relationships between *Dendrobium* and *Bulbophyllum* from mainland India and associated islands were not derived.

According to the study of host plant preference plant families; Myrtaceae, Lauraceae and Euphorbiaceae act as suitable hosts for orchids of *Dendrobium* and *Bulbophyllum*. However, *Dendrobium* and *Bulbophyllum* species are found to be dominated (25%) on tree barks of genus *Syzygium*. But the genus *Glochidion* (family Euphorbiacea) is the preferred host for *Dendrobium* species whereas genus *Syzygium* (family Myrtaceae) is the preferred host for most *Bulbophyllum* species.

In conclusion, findings of the present research would undoubtedly contribute to the progression of the taxonomy of the studied taxa and developing conservation strategies. Further, these findings would be significant in quality improvements of those orchids as novel floriculture products.

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BIOFILM ENRICHED EPPAWALA ROCK PHOSPHATE AS A POTENTIAL PHOSPHORUS FERTILIZER TO REPLACE TRIPLE SUPER PHOSPHATE IN RICE CULTIVATION

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Rice (*Oryza sativa L.*) is one of the dominant food crops grown in Sri Lanka. It occupies approximately 34% (0.77 million ha) of total cultivated lands in Sri Lanka (Ministry of Agriculture, 2005). On average, 560,000 ha in wet season and 310,000 ha in dry season are cultivated to reach average annual extent of about 870,000 ha of rice cultivation. Most farmers use chemical fertilizers (CF) as a nutrient source in rice cultivation. Urea, Triple Super Phosphate (TSP) and Muriate of Potash (MOP) are the commonly applied CFs in rice cultivation (Ministry of Agriculture, 2005). Amongst them TSP is used as the main source of phosphorous (P) in rice cultivation due to its high solubility for short term fast-growing crops such as rice. However, TSP application leads to serious environmental issues such as accumulation of trace elements (Aluminum, Chromium, Nickel, Cadmium, Lead, and Uranium) in agricultural fields (Chandrajith *et al*., 2011). Therefore, it is important to find an alternative for replacing TSP in agricultural fields. Eppawala Rock Phosphate (ERP) is considered as a cheap and environmental-friendly alternative to TSP, despite few constraints like low solubility which limits the direct application of it as a P fertilizer to few perennial crops such as tea, rubber, coconut, and export agricultural crops. It has been reported that microbial interventions like developed biofilms can increase P solubilization of ERP (Jayaneththi *et al.,* 2021). Therefore, this study is focused on testing the ability of replacing TSP by applying different rates of ERP enriched with biofilms in rice cultivation.

Objective

The objective of this research was to develop a biofilm-enriched ERP as an alternative P source for TSP in rice cultivation.

Methodolgy

A field experiment was carried out in a farmer field at Puliyankulama in Anuradhapura, which belongs to the DL1b agro-ecological region with an average annual temperature of 27 °C and average annual rainfall of 1,368

mm during dry and wet seasons. Fields were ploughed, harrowed and leveled according to the recommendations by the DOA. Rice variety BG 352 (3.5 months old) which is the most popular rice variety in the area was used. Crop was established by broadcasting the water-soaked pre-germinated seeds at the recommended rate. The experiment was arranged in a Randomized Complete Block Design (RCBD), with three replicates. The plot size was 3 m × 6 m. The initial properties of the soil collected from the field site was anlysed.

There were five treatments with a no amendment control (Table 1). The CF dosage recommended by the DOA in 2013 for rice (CF_E, 340 kg ha^{-1} of urea, TSP and MOP) was modified by replacing TSP (55 kg ha^{-1}) with BEE (92 kg ha^{-1}) (CF_M) on the basis of P content. A developed P solubilizing biofilm (Jayaneththi *et al.,* 2021) was sprayed on ERP at the rate of 1.7 L per 100 kg as recommended by the NIFS for preparation of BEE. In addition, Biofilm biofertilizers (BFBF) which reduces the use of CF in rice cultivation (Premarathna *et al.,* 2021) was coupled to the treatments.

Table 1. Treatments applied to the field experiment

Treatments
100% CF_E (T1)
CF_M L1 + BFBF (T2)
CF_M L2 + BFBF (T3)
CF_M L3 + BFBF (T4)
100% CF_{ENK} (only N, K) (T5)
Control (soil alone) (T6)
CF_M (L1) - Urea (65%) MOP (65%) BEE (100%)
CF_M (L2) - Urea (65%) MOP (65%) BEE (80%)
CF_M (L3) - Urea (65%) MOP (65%) BEE (65%)

Following the DOA (Amarathunga *et al.,* 2018) and NIFS recommendations (Premarathna *et al.,* 2021) the inorganic and BEE treatments were broadcasted into plots since all fertilizers are in solid-based forms. All management practices such as weeding, pest and disease management etc. were carried out according to the recommendations given by the DOA.

At the harvest of the crop, root and shoot dry masses, panicle length and plant height were measured by taking five random plants excluding the boarder rows from each plot. Numbers of filled grains and unfilled grains per panicle were counted of the five plants. Plots were harvested excluding the borders in each sub plot (1 x 1 m) and grain yield was recorded after air drying.

Results and Discussion
Initial soil properties
The surface soils of the experimental site was clay in texture and classified as very poorly drained Reddish Brown Earth (Panabokke, 1996) that consisted of low organic matter (1.65%) in neutral soil reaction (pH = 7.3). It had a moderate level of available N. Available P content was close to critical or deficient level while K was at the sufficient level, but not in the optimum range for rice plant growth (Portch and Hunter, 2002). Soil microbial biomass was also very low initially.

Plant growth parameters: Root & shoot dry mass, panicle length and plant height
The CF_M L1 + BFBF treatment with 100% BEE, performed better than the other treatments for the measured plant growth parameters (i.e. root & shoot dry mass, panicle length and plant height). For root & shoot dry mass in both seasons, CF_M L1 + BFBF showed the significantly *(p<0.05)* highest performance while the significantly *(p<0.05)* lowest was recorded in the control. However, the average plant growth in the dry season was lower than that of the wet season due to a drought condition. Following the similar pattern recorded for root & shoot dry mass in the both seasons, the significantly *(p<0.05)* highest plant height and panicle lengths were recorded in CF_M L1 + BFBF. This was due to the significant role played by microbial biofilms in enhancing plant growth by secreting plant growth promoting (PGP) substances (Bandara *et al*., 2006), which eventually result in increasing nutrient uptake in comparison to synthetic fertilizers. Further, application of biofilms to soil under suitable conditions triggers them to secrete metabolites, acids and enzymes, which make deficient nutrients readily available for plants (Meena *et al.* 2015).

Yield parameters: Filled & un-filled grain weights and grain yield

In both seasons, CF_M L1+ BFBF recorded the highest filled grain weights and the lowest un-filled grain weights.

Generally, soil biofilms are plant growth promoters that stimulate the growth through a range of mechanisms improving nutrient acquisition and inhibition of fungal plant pathogens (Biro *et al.,* 2000). In the present study, the developed biofilm (Jayaneththi *et al.,* 2021) was composed of nitrogen fixing and P solubilizing bacteria and root-associated fungi, which contributed to improved plant nutrition (Weerasundara *et al.,* 2014), eventually producing the highest yield both in dry (1884 kg ha^{-1}) and wet (6181 kg ha^{-1}) seasons (Table 2) with the treatment of CF_ML1+ BFBF. The control plots recorded the significantly *(p<0.05)* lowest yield in both seasons (1119 kg ha^{-1} in dry and 3312 kg ha^{-1} in wet). However, the overall grain yield of the dry season was below the average yield because of the drought condition existed during the season.

Several other studies also reported that the use of the developed biofilms enhanced the growth, yield, and quality of the product in many crops (Buddhika *et al.*, 2016). As identified by Kundu *et al.* (2013), the application of P solubilizing fungal-bacterial biofilm enhanced the yield of green gram and maize (Umesha *et al.,* 2014).

Table 2. Grain yields at the harvest of dry (2018) and wet (2018/2019) seasons.

Treatment	Yields	
	Dry season (2018) (kg ha^{-1})	**Wet season (2018/2019) (kg ha^{-1})**
T1	1710 ± 11[b]	5632 ± 6 [b]
T2	1884 ± 23[a]	6181 ± 9[a]
T3	1881 ± 16[a]	5740 ± 12[b]
T4	1705 ± 2[b]	5190 ± 8[c]
T5	1837 ± 13[a]	5283 ± 4[c]
T6	1119 ± 17[c]	3312 ± 6[d]

Different letters in each column are indicated the statistically significant differences at 5% probability level according to the Tukey's mean comparison test.

Conclusion

This experiment revealed the potential of cutting off the full dosage of TSP-P by BEE-P, which achieved the yield levels similar to the DOA-recommended TSP dosage in 2013. The results suggested that 65% of CF Urea and MOP (CF_M) was sufficient with the 100% BEE to replace the TSP-P. This short-term study revealed that the yields are not hampered by replacing TSP-P from BEE-P. However, long-term experimental results are required to develop more reliable recommendations.

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IMPROVED ESTIMATION WITH INCOMPLETE PRIOR INFORMATION IN MISSPECIFIED REGRESSION MODEL

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Misspecification of the linear regression model is inevitable when fitting statistical models. Model misspecification due to omitting important explanatory variables from the model is considered as a major issue, which violates the assumptions of the linear regression model, and leads to unreliable estimation. The Ordinary Least Square Estimator (OLSE) does not retain its desirable properties if multicollinearity exists among explanatory variables. Biased estimators such as Ridge Estimator (RE), Almost Unbiased Ridge Estimator (AURE), Liu Estimator (LE), Almost Unbiased Liu Estimator (AULE), Principal Component Regression Estimator (PCRE), r-k class estimator, and r-d class estimator have been used in the literature to resolve this issue. Furthermore, the parameter estimation can be strengthened if prior knowledge about the regression coefficient is applied. Many researchers proposed stochastic restricted estimators such as Mixed Regression Estimator (MRE), Stochastic Restricted Ridge Estimator (SRRE), Stochastic Restricted Almost Unbiased Ridge Estimator (SRAURE), Stochastic Restricted Liu Estimator (SRLE), Stochastic Restricted Almost Unbiased Liu Estimator (SRAULE), Stochastic Restricted Principal Component Regression Estimator (SRPCRE), Stochastic Restricted r-k class estimator (SRrk), and Stochastic Restricted r-d class estimator (SRrd) to incorporate prior information to the regression model. However, these estimators yield high bias when the number of explanatory variables is high, and they do not care about irrelevant variables while fitting models. The Least Absolute Shrinkage and Selection Operator (LASSO) has been widely used in recent research to tackle this issue. Since LASSO is unstable under a strong multicollinearity problem, Elastic Net Estimator (ENet) was introduced as an improved solution. In addition, the adaptive LASSO estimator is also used to improve the consistency of variable selection. The LARS algorithm has been used to obtain these estimators' solutions. The objectives of this PhD research were (I) to examine the performance of estimators based on the sample information in the misspecified linear

regression model, (II) to examine the performance of estimators based on the sample information and prior information about regression coefficient in the misspecified linear regression model, (III) to propose new estimators by studying the stochastic properties of estimators considered in this study for the misspecified linear regression model, and (IV) to improve the parameter estimation in the high dimensional linear regression model.

Methodology
This research adopted a theoretical approach.
1. Guided essential reading was undertaken relevant to the research problem.
2. An analytical study of relevant research articles was done, and findings were summarized and interpreted further to define a theoretical framework for this research.
3. The relevant biased/stochastic restricted estimators were used in the misspecified regression model, and comparisons were made with the other alternative estimators.
4. A simulation study was done to justify the theoretical findings, and the validity of the results was checked by applying real-world data.

Results and Discussion
Consider the linear regression model
$$y = X\beta + \varepsilon, \qquad (1)$$
where X is $n \times p$ matrix of explanatory variables, β be the $p \times 1$ vector of unknown coefficients, and ε be the $n \times 1$ vector of disturbances such that $\varepsilon \sim N(0, \sigma^2 I)$. Now we rewrite the correct model (1) as
$$y = X_1\beta_1 + X_2\beta_2 + \varepsilon \qquad (2)$$
where X_1 and X_2 are the $n \times l$ and $n \times p$ matrices of observations on the $m = l + p$ regressors, β_1 and β_2 are the $l \times 1$ and $p \times 1$ vectors of unknown coefficients. Let us assume that X_2 is the most influential variable matrix, and the researcher constructed the model (2) without X_2 as
$$y = X_1\beta_1 + u \qquad (3)$$
where $u = X_2\beta_2 + \varepsilon$. The model (3) is called as a misspecified regression model due to excluding some relevant explanatory variables from the model. To get simplified expressions, we apply spectral decomposition to the symmetric matrix $X_1'X_1$. Then we have $T'X_1'X_1T = \Lambda$, where $\Lambda = diag(\lambda_1, \ldots, \lambda_l)$ and $T = (t_1, t_2, \ldots, t_l)$ is

the orthogonal matrix and $\lambda_i > 0$ is the ith eigenvalue of $X_1'X_1$. Let $T_h = (t_1, t_2, \ldots, t_h)$ be the remaining columns of T having deleted $l - h$ columns where $h \leq l$. Hence, $T_h'X_1'X_1T_h = \Lambda_h = diag(\lambda_1, \ldots, \lambda_h)$. Let $Z = X_1T$ and $\gamma = T'\beta_1$. Then the models (2) and (3) can be written in canonical form as
$$y = Z\gamma + \delta + \varepsilon \qquad (4)$$
$$y = Z\gamma + u \qquad (5)$$
respectively, where $\delta = X_2\beta_2$. Then, we proposed a generalized form to represent the estimators OLSE, RE, AURE, LE, AULE, PCR, r-k class estimator and r-d class estimator for the model (5) as
$$\hat{\gamma}_G = G\Lambda^{-1}Z'y \qquad (6)$$
where

$$\hat{\gamma}_G = \begin{cases} \hat{\gamma}_{OLSE} & \text{if } G = I \\ \hat{\gamma}_{RE} & \text{if } G = (\Lambda + kI)^{-1}\Lambda \\ \hat{\gamma}_{AURE} & \text{if } G = (I - k^2(\Lambda + kI)^{-2}) \\ \hat{\gamma}_{LE} & \text{if } G = (\Lambda + I)^{-1}(\Lambda + dI) \\ \hat{\gamma}_{AULE} & \text{if } G = (I - (1 - d)^2(\Lambda + I)^{-2}) \\ \hat{\gamma}_{PCRE} & \text{if } G = T_hT_h' \\ \hat{\gamma}_{rk} & \text{if } G = T_hT_h'(\Lambda + kI)^{-1}\Lambda \\ \hat{\gamma}_{rd} & \text{if } G = T_hT_h'(\Lambda + I)^{-1}(\Lambda + dI) \end{cases}$$

having the shrinkage parameters $k > 0$ and $0 < d < 1$.

Let us also assume that there exist prior information on β_1 for model (3) in the form of
$$\varphi = R\beta_1 + g + v \qquad (7)$$
where φ is the $q \times 1$ vector, R is the given $q \times l$ matrix with rank q, g is the $q \times 1$ unknown fixed vector, v is the $q \times 1$ vector of disturbances, such that $E(v) = 0$, $D(v) = E(vv') = \Psi = \sigma^2 W$; W is positive definite, and $E(vu') = 0$. Now we apply the simultaneous decomposition for the symmetric matrices $X_1'X_1$ and $R'\Psi^{-1}R$ where $X_1'X_1$ is a positive definite matrix, and $R'\Psi^{-1}R$ is a positive semi-definite matrix. Then, we have
$$B'X_1'X_1B = I \quad \text{and} \quad B'R'\Psi^{-1}RB = \Lambda_*.$$
where B is a $l \times l$ nonsingular matrix, Λ is a $l \times l$ diagonal matrix with eigenvalues $\lambda_i > 0$ for $i = 1, 2, \ldots, q$ and $\lambda_i = 0$ for $i = q + 1, \ldots, l$. Let $Z_* = X_1B$, $R_* = RB$, $\gamma_* = B^{-1}\beta_1$, $Z_*'Z_* = I$ and $R_*'\Psi^{-1}R_* = \Lambda_*$. Then the models (2), (3) and (7) can be written as

$$y = Z_*\gamma_* + \delta + \varepsilon \qquad (8)$$
$$y = Z_*\gamma_* + u \qquad (9)$$
$$\varphi = R_*\gamma_* + g + v \qquad (10)$$

respectively. Then, we proposed a generalized form to represent the stochastic restricted estimators MRE, SRRE, SRAURE, SRLE, SRAULE, SRPCR, SRrk and SRrd for the model (9) as

$$\widehat{\gamma}_G^* = G_*(I + \sigma^2 \Lambda_*)^{-1}(Z_*'y + R_*'W^{-1}\varphi) \quad (11)$$

where,

$$\widehat{\gamma}_G^* = \begin{cases} \hat{\gamma}_{SRRE} & \text{if } G_* = 1 \\ \hat{\gamma}_{SRRE} & \text{if } G_* = (1+k)^{-1} \\ \hat{\gamma}_{SRAURE} & \text{if } G_* = (1+k)^{-2}(1+2k) \\ \hat{\gamma}_{SRLE} & \text{if } G_* = 2^{-1}(1+d) \\ \hat{\gamma}_{SRAULE} & \text{if } G_* = 2^{-2}(1+d)(3-d) \\ \hat{\gamma}_{SRPCRE} & \text{if } G_* = T_h T_h' \\ \hat{\gamma}_{SRrk} & \text{if } G_* = (1+k)^{-1}T_h T_h' \\ \hat{\gamma}_{SRrd} & \text{if } G_* = 2^{-1}(1+d)T_h T_h' \end{cases}$$

having $k > 0$ and $0 < d < 1$ as shrinkage parameters. Further, we proposed the Sample Information Optimal Estimator (SIOE) by studying the propertied of the biased estimators as

$$\widehat{\gamma}_{SIOE} = G_{opt}\Lambda^{-1}Z'y \qquad (12)$$

where
$G_{opt} = \frac{1}{2}\big((\gamma + \Lambda^{-1}Z'\delta)\gamma' + \gamma(\gamma + \Lambda^{-1}Z'\delta)'\big)(\sigma^2\Lambda^{-1} + (\gamma + \Lambda^{-1}Z'\delta)(\gamma + \Lambda^{-1}Z'\delta)')^{-1}$. Also, we proposed the Stochastic Restricted Optimal Estimator (SROE) by studying the propertied of the stochastic restricted estimators as

$$\widehat{\gamma}_{SROE} = G_{opt}^*(I + \sigma^2\Lambda_*)^{-1}(Z_*'y + R_*'W^{-1}\varphi) \tag{13}$$

where $G_{opt}^* = \frac{1}{2}\Big(\big(\gamma_* + (I + \sigma^2\Lambda_*)^{-1}(Z_*'\delta + R_*'W^{-1}g)\big)\gamma_*' + \gamma_*\big(\gamma_* + (I + \sigma^2\Lambda_*)^{-1}(Z_*'\delta + R_*'W^{-1}g)\big)'\Big)\Big(\sigma^2(I + \sigma^2\Lambda_*)^{-1} + \big(\gamma_* + (I + \sigma^2\Lambda_*)^{-1}(Z_*'\delta + R_*'W^{-1}g)\big)\big(\gamma_* + (I + \sigma^2\Lambda_*)^{-1}(Z_*'\delta + R_*'W^{-1}g)\big)'\Big)^{-1}$.

The performance of the biased estimators and stochastic restricted estimators were examined using Monte-Carlo simulation and using a real-world example in Scalar Mean Square Error (SMSE) criterion. Among the existing biased estimators, SIOE showed the superiority in SMSE criterion when multicollinearity exists, even in the misspecified regression model due to omitting significant explanatory variables. Among the existing stochastic restricted estimators,

SROE showed better performance under the misspecified regression model when multicollinearity exists in the SMSE criterion.

In the case of high dimentional regression model, the LASSO is defined as
$$\widehat{\beta}_{LASSO} = \underset{\beta}{\text{argmin}}\{(y - X\beta)'(y - X\beta)\}$$
subject to $\sum_{j=1}^p |\beta_j| \le t$, $\qquad$ (14)
where $t > 0$ is a turning parameter and, the adaptive LASSO is defined as $\widehat{\beta}_{adpLASSO} = \underset{\beta}{\text{argmin}}\{(y - X\beta)'(y - X\beta)\}$ subject to
$$\sum_{j=1}^p w_j |\beta_j| \le t, \qquad (15)$$
where w_j is a weight vector. Then, we proposed the GLARS to combine LASSO with other biased estimators, SRGLARS to combine LASSO with other stochastic restricted estimators, Adaptive GLARS to combine adaptive LASSO with other biased estimators, and Adaptive SRGLARS to combine adaptive LASSO with other stochastic restricted estimators for model (1) as outlined below:

Algorithm 1: GLARS/SRGLARS/Adaptive GLARS/Adaptive SRGLARS

1: Standardize X to have a mean zero with a standard deviation of one, and center the y to have a mean zero.
2: Start with all estimates of the coefficients $\widehat{\beta} = 0$ with the residuals $r = \hat{\varepsilon}$ and $\tau = \hat{v}$.
3: Find the predictor X_j most correlated with r; $j = 1, 2, \ldots, p$.
4: Move the estimate of $\hat{\beta}_j$ from 0 towards the $\widehat{\beta}_G$ direction based on GLARS/SRGLARS/ Adaptive GLARS/ Adaptive SRGLARS until some other predictor X_k has as large a correlation with the current residual as X_j does.
5: Move $\hat{\beta}_j$ and $\hat{\beta}_k$ in the direction defined by their joint $\widehat{\beta}_G$ direction of the current residual on (X_j, X_k), until some other predictor X_l eventually earns its way into the active set.
6: If a non-zero coefficient hits zero, drop its variable from the active set of variables and recomputed the current joint $\widehat{\beta}_G$ direction.
7: Repeat the steps 5 and 6 until required conditions attained.

The GLARS/SRGLARS/ Adaptive GLARS/Adaptive SRGLARS algorithm's different combinations of LASSO and biased

and stochastic restricted estimators were examined using the Root Mean Square Error (RMSE) criterion. Among the different combinations, GLARS outperforms when it combines LASSO with SIOE, adaptive GLARS outperforms when it combines adaptive LASSO with SIOE, SRGLARS outperforms when it combines LASSO with SROE, and adaptive SRGLARS outperforms when it combines LASSO with SROE in the RMSE criterion. We referred to these combinations as LARS-SIOE, LARS-SROE, adaptive LARS-SIOE, and adaptive LARS-SROE, respectively.

Conclusion

This research presented the simplest ways to understand the several biased estimators and stochastic restricted estimators very easily using the generalized forms. The SIOE and SROE can be used for the regression model's parameter estimation instead of considering several biased and stochastic restricted estimators, where SROE uses the prior information about regression coefficients while fitting the models. In high-dimensional regression model, adaptive LARS-SROE shows better performance in the RMSE criterion when correct prior information is used. Also, adaptive LARS-SROE showed the superiority in the mis specified regression model, even in the case of incomplete prior information. We clearly showed the improvement of estimators or algorithms' prediction performance when prior information of regression coefficients is used. In practice, model misspecification and the use of incomplete prior information are unavoidable, and this research suggested the most suited estimators and algorithms for these cases.

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FUZZY INFERENCE MODEL FOR EMPLOYEE PERFORMANCE APPRAISAL FOR CENTRAL GOVERNMENT OF SRI LANKA

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Employees play a vital role in an organization and they are a valuable asset to the organization. Performance appraisal involves identifying and evaluating the performance of the employees with the aim of effectively rewarding their efforts to motivate them towards incessant pursuit of organizational objectives. Managing the human resource effectively is important for both private and public organizations to reach their goals. The measurement of employees' performance is also important for the development of an organization. Managing human resources is an essential matter for an organization to get the maximum contribution from the employees. The employee appraisal system is designed to measure the employee performance based on certain identified factors, which will allow the employer to identify the performance level of each employee. The performance of an employee is usually measured against factors such as job knowledge, leadership ability, personal skills, quality of work, versatility among others, etc.

The appraisal systems are designed based on the organization culture and the objectives. These systems benefit not only the employer but also the employees. Employees are informed about the expectations of the employer and the individual performance levels. The main goal of the system is to assess the performance level of each employee and get the maximum contribution from them to the organization. Employees' performance directly influences the overall performance of an organization. This is one of the reasons why the appraisal systems to measure the performance of employees become popular in any organization. The performance evaluation is not an easy task, because it needs to consider a variety of factors affecting the employees as well as the organization. There are two types of measures in performance appraisal; objective measures and subjective measures. Most of the performance appraisal systems use the combination of these measures. Performance appraisals are mainly used for judgmental and developmental purposes and to make good administrative decisions for an organization.

The objectives of the present study were,

(I) To design a model to identify performance level of the employees in Accounts Divisions in Central Government of Sri Lanka.

(II) To recognize the priority factors affecting the employee performance level in Accounts Divisions in Central Government of Sri Lanka.

Methodology
This Fuzzy inference model is a multi-input single-output Mamdani type Fuzzy inference system. The input variables were classified as Adopt to Organization Rules (AOR), Work Achievement (WA), Subject Knowledge (SK) and Inter-Relationship among Employees (IRE). A well-defined questionnaire was used to collect data from the users. The responses to the questions were analyzed and converted to Fuzzy inputs to this inference system.

These Fuzzy inputs were used to compute the employee performance appraisal level. The triangular membership function was used to determine the values of these input variables.

Questionnaire was used to collect primary data from the users. Well defined questionnaire helped to get more precise data from them. Here, the questionnaire was constructed with the assistance of the subject experts and based on the information collected from the literature review. An important phase of the research was to prepare a simple, user friendly and understandable questionnaire to get the reliable data from the users. As this system is to identify the performance level of the government employees, the questionnaire was designed according to the subject and got the data in all the categories related to the employee. Appraiser had to select the relevant option out of the three options; 'NO', 'AVG' and 'YES' for each question related to the employee.

This model introduced a new value called Degree of Rate (Deg.Rate). Here, the Fuzzy set values "NO", "AVG" and "YES" were assigned the degree of rate "1", "2" and "3" respectively. The Fuzzy rules were used for the decision making purpose and the number of rules depended on the number of inputs and the number of fuzzy set values of the system.

Each question item in the questionnaire was assigned a weight. This weight for the question was determined based on the information from the domain experts. For each rule, the product of the weight of the variable and the degree of rate was calculated. The summation of these products output the antecedent value of a rule.

This model used minimum and maximum antecedent values to define the antecedent range. Here, the minimum value was 1 and the maximum value was 3. The antecedent range was denoted as [1,3]. Based on this range, the decision rage was defined as [10,90]. This decision value was used to define the final membership functions. For the simplicity, triangular membership functions were used. All the generated MFs were input to Mamdani Fuzzy inference model. Here, Centroid defuzzification method was used to convert the Fuzzy output values to crisp values. Finally, the numerical value was calculated to identify the employee performance level.

Results and Discussion
The employees got the result as "Fair" in many cases in the current process, because the evaluator did not like to give marks and appreciate his employees. It is a psychological issue, and it may badly affect the employee as well as the organization. Paper base process gives less effectiveness and an annually updating systematic process can be used to review an employee in a much effective manner. The current process did not give marks to the employees. Because of that the employees got the output as "Fair" and they were not trying to give their full strength and the capacity for the duties. The traditional method and the statistical method were used for the results comparison. Mainly, the traditional method was used to compare the proposed novel method with the methods that are used in the common structure in current performance evaluation. Those values can be used by the domain experts to evaluate the output of the Fuzzy Inference method with the current process.

Conclusion
Employee performance appraisal process gives benefits to employees as well as to the organization. Fuzzy logic is considered as the most powerful tool for performance appraisal method. The traditional paper based method

was used to evaluate performance in Central Government of Sri Lanka. The employees in accounts sections in Central Government of Sri Lanka carried out variety of tasks and they are not satisfied with the evaluation of their performance at the office. The current performance evaluation is the traditional manual process and it has drawbacks to employee as well as to the employer. The true assessment of the employee performance could not be evaluated using this current process.

Pre-survey was conducted to identify problem and it reflected 74% of the selected employees were disagree with the current process. Through literature review and discussion with the domain experts, independent variables for the proposed model were identified. Reliability and validity tests were carried out to identify the suitable variables to evaluate performance index.

Traditional methods and statistical methods were used to compare the results with this novelty model. By comparing the model with the traditional methods, this model output a realistic value for the employee based on their contribution to the work. The statistical view of the output of the model pointed out that there is a strong positive relationship between the independent variables and the performance index. Also, this model is statistically significant and good fit to data. The proposed Fuzzy inference model was expected to evaluate the employee performance by using a graphical user interface to give a user friendly environment. Flexibility of this model allows decision makers to take decisions easily. First, employees need to have a clear view on the assessment process of their performance. Better understanding of the process gives benefits to the organization as well as to the employees.

This model can also be used as a tool in periodic evaluations requested by the institute. Usually, annual internal evaluations are performed. By using this model, employer can evaluate the employees and their results can be used to determine next year's tasks of the section.

This model used four criteria to define performance of employee. They are; adopt to organization rules, work achievement, subject knowledge and inter relationship among employees. Weights assigned for each variable identify the priority factors that used to evaluate the performance. This method gives more realistic performance level than the traditional paper based evaluation. Graphical interface encourages the user to utilize the system to do performance evaluation.

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GESTURE AND PEDESTRIAN BEHAVIOR ANALYSIS FOR SAFE VEHICLE NAVIGATION

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Autonomous vehicles can directly impact society both at micro and macro levels (Winkle, 2016) and they require the ability to communicate with pedestrians and traffic police officers to understand their intentions. Besides being a dynamic control task, driving is a social phenomenon that requires a mutual understanding between fellow road users involved, in order to ensure the smooth flow of traffic and to guarantee the safety of everyone (Rasouli *et al*., 2018). Traffic on roads, driving conditions, traffic police officers and their signals, and various behaviours of pedestrians can vary from country to country. Understanding pedestrian behaviour and their signals, and traffic police signals, however, is not intuitive and depends on various factors such as demographics of the pedestrians, traffic dynamics, environmental conditions, etc. Depending on the country and the situation, pedestrians sometimes cross the road by using hand signals to stop traffic. Also, traffic police officers may control vehicles during traffic jams, traffic light malfunctions, and zebra crossing lines. The common signals they use are STOP or GO. Convolutional Neural Networks (CNN), a deep learning technology, is being used in several different areas such as computer vision and object recognition. This study focuses on how an AV can identify the STOP signal shown by a pedestrian or a traffic police officer by using their hand among other pedestrians and traffic police officers on the road. The proposed model can identify human hand signals and pedestrian crossing lines. We designed a small size automated vehicle using a Raspberry Pi. This prototype AV was used to test the proposed model for its real-time operation. The AV can detect five classes of objects and move forward or stop considering the relevant signals. The traffic conditions of Sri Lanka were considered for this study since the case study was done in Sri Lanka.

The objectives of the present study were,

(I)   To create a multi-class Object detection model which can be used to detect pedestrians and police gestures and hand signals

(II) To deploy the models in real-time driving conditions using a prototype car and test the best performance model.

Methodology

The proposed methodology contains five steps including data collection, data preprocessing, object detection models selection, and finally designing a prototype car to implement the results as shown in Figure 1. Each step is described in detail below.
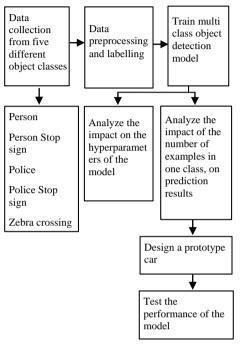

Figure 1: Methodology

The study considers images from five different object classes which are common on the road. The names and descriptions of the five object classes are listed in Table 1. Data sets need to contain all the possible background and lighting conditions and different environments such as local and international. Therefore, data were collected in three different scenarios. Those are indoor collected data, outdoor collected data and extracted images from the Joint Attention in Autonomous Driving data set. Then the collected images from each approach are named as Data Set 01, Data Set 02 and Data Set 03. Data Set 01 consists of images collected in indoor environments. Six members participated in the data collection and the members of the group acted according to the gestures and signals wanted. All the images were collected from 2m, 4m and 7m distances from the cameras. This data set contains images from three classes as Person, Person Stop and Police Stop.

Table 1. Object classes in the data sets

Object Class	Description
Person	Person class represents pedestrians on the roads
Person Stop	Person Stop class includes images of pedestrians signaling to stop moving vehicle
Police	Police class includes images of traffic police officers on the roads
Police Stop	Police Stop class contains images of traffic police officers gesturing to stop traffic
Zebra crossing	Zebra crossing class includes images of pedestrian crossing lines on the roads

Data Set 02 was collected as video recordings during real-time driving in road conditions. Then by using FFmpeg (Tomar & Suramya, 2006), images were extracted from the video files, 3,383 images were extracted by using this method. There are images for all five different classes. Those are Person, Person Stop, Police Stop, Police, and Zebra crossing.

To include images from the international road conditions as well as to analyze the impact of the number of images in each class on the final results of the model, images from the Joint Attention in Autonomous Driving (JAAD) data set were selected (Fang & López, 2018). This dataset was named Data Set 03.

Among several multi-class object detection models, Faster R-CNN was selected as the object detection model for this study. Also, for comparison purposes of the performance and complexity of Faster R-CNN, the other model we used was SSD. Precisely, Faster R-CNN introduced the Region Proposal Network (RPN). In the last layer of an initial CNN, a sliding window size of 3×3 moves across the feature map and maps it to a lower dimension as shown in Figure 2. The training data set was generated manually by tagging the classes in the images using LabelImg (Tzutalin, 2015). Then from the selected images, 80% were taken for training and 20% for the test. Precision, recall, F1-score and mAP (Mean Average Precision) were used as the evaluation metrics for the Models.
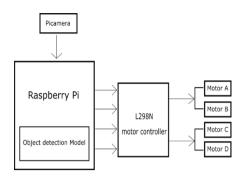
Figure 2: Circuit diagram of the prototype car

A prototype car is developed to test the proposed solution. Raspberry Pi 3 Model B has been used as the microcontroller. The front and rear motors of the car are controlled by the L298N motor controller. The complete circuit diagram of the prototype car is presented in Figure 3.
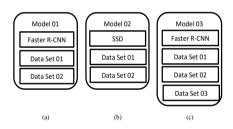

Figure 3: Models with their trained data sets, Model 01 (a), Model 02 (b), and Model 03 (c)

Results and Discussion

Three experiments were conducted with Faster R-CNN along with SSD which was used for comparison purposes. SSD is another multi-class object detection model. SSD simultaneously predicts the bounding box and the class as it processes the image in a single shot. Faster R-CNN and SSD with Data Set 01 and Data Set 02 were considered as Model 01 and Model 02. To find the effect of the number of images in a class for the final performance of the model, Faster R-CNN was trained with Data Set 01, Data Set 02 and Data Set 03. This approach was named Model 03. Models and selected data sets are represented in Figure 4. Trained models were used for testing (that is

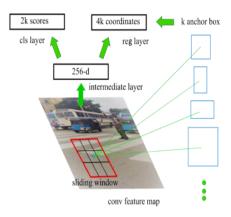verification) with the specially created data set with 238 images.

Figure 4: Faster R-CNN feature map

After 200,000 epochs of each model, Model 03 predicted classes with the highest performance. The performance of Model 03 is presented using Precision, Recall and F1-score value are presented below in Table 2. The main reason for the better performance of Faster R-CNN was its use of region proposal networks.

Table 2: Precision and recall of Model 03

	Person	Person Stop	Police	Police Stop	Zebra crossing	Avg
Precision	0.93	0.57	0.66	0.91	0.93	0.80
Recall	0.97	0.65	0.78	0.95	0.89	0.84
F1-score	0.94	0.60	0.71	0.92	0.90	0.81

Conclusion

For navigation of a self-driving car, several areas that have to be considered, when it is implemented in different traffic situations such as on roads where pedestrians randomly cross the road or traffic police officers control the traffic. To solve this issue multi-class object detection models were designed to detect pedestrian and traffic police officers and their hand signals. The images were collected in five different classes. Those are Person, Person stop, Police stop, Police, and Zebra crossing.

By considering all the test results and performance, it was concluded that the Faster R-CNN is the most accurate model compared to the other two models. The number of images in the data set and the quality of the images was

one of the factors that can cause the sensitivity of the model accuracy. It is recommended to use images with high sharpness and resolution. The large size of the images can result in a slower training time for the models. Finally, a Raspberry Pi powered prototype car was designed to deploy the models. Each model was tested with the prototype car and the best model for real-time driving conditions was investigated. Picamera had a better-quality camera which was used to take live video feeds and it is lightweight. Therefore, Picamera is recommended for any devices like Raspberry Pi and projects that need a better lightweight camera.

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DIVERSITY, DISTRIBUTION, BREEDING PREFERENCEAND THE VECTOR POTENTIAL OF ORNITHOPHILIC MOSQUITOES IN SRI LANKA

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Wild birds have been identified as the reservoirs of many pathogens in nature that are transmissible to humans and other wildlife through mosquitoes that preferentially feed on birds (ornithophilic mosquitoes). These mosquitoes have been documented as competent vectors of several devastating bird diseases (*i.e.* West Nile Virus, St. Louis Encephalitis, Western Equine Encephalomyelitis, Eastern Equine Encephalomyelitis) that transmit from birds to birds as well as from birds to humans and other animals through their bites (Farajollahi *et al.*, 2011). Vector potential of ornithophilic mosquitoes has also been recognized for avian haemoparasites including *Haemoproteus* spp., *Plasmodium* spp., and *Leucocytozoon* spp. that are transmitted only from birds to birds (Valkiūnas, 2005). The majority of reported ornithophilic mosquitoes belong to the genus *Culex* (Molaei*et al*., 2010). Mosquitoes of the genera *Aedes*, *Aedeomyia*, *Anopheles*, *Coquillettidia*, *Mimomyia*, *Tripteroides*, *Mansonia*, *Uranotaenia*, and *Verrallina* have also been recognized as ornithophilic mosquitoes (Jansen *et al*., 2009). However, there are many gaps in understanding mosquito-bird interactions and the transmission of disease-causing pathogens through ornithophilic mosquitoes. This is an emerging field of study in Sri Lanka.

Sri Lanka is a tropical island and a biodiversity hotspot rich with high endemism. Due to the high diversity of birds (506 species) and mosquitoes (known as 159 species) in the country, there are ample opportunities to study the ecological interactions and relationships between mosquitoes and birds. Being an attractive migratory destination for winter migrant birds that come from all over the world, there are opportunities for novel pathogens to reach the country when those birds act as reservoir hosts. There are opportunities for those pathogens to infect local bird populations, other wildlife, and humans in the country. Mosquitoes that feed on those birds can harbor and transmit many viruses and blood parasites to residential birds, wild animals, and humans. Thus, the role of bird-biting mosquitoes in disease transmission is an important aspect that needs further

elucidation. The investigation of vector-borne pathogens in local bird communities and the risk of invasion of new pathogens to and from other countries through migratory birds are also crucial in understanding the risk of emerging infectious diseases and the enzootic and zoonotic capabilities of those pathogens. Due to the lack of such studies, there are gaps in understanding the diversity, abundance, distribution, activity pattern as well as breeding sites of ornithophilic mosquitoes. Thus, this study was designed to explore ornithophilic mosquitoes in Sri Lanka and to understand the vector potential of those mosquitoes.

The objectives of the study were,

(I) To determine the abundance, diversity, distribution, and activity pattern of ornithophilic mosquitoes in Sri Lanka.

(II) To identify the spatial distribution of ornithophilic mosquitoes in Sri Lanka.

(III) To determine the potential vector status of ornithophilic mosquitoes.
   a) Pathogens present in the blood of the local and migratory bird populations.
   b) Pathogens present in the mosquito populations.

(IV) To determine the common breeding places and coexisting patterns of ornithophilic mosquitoes in Sri Lanka.

Methodology
The study was conducted from November 2016 to December 2019 covering highland forests, lowland forests, and human settlements.

Objective I: Activity pattern of ornithophilic mosquitoes was determined by a preliminary study using 24 hours mosquito sampling efforts at two habitat types in Gampola, Sri Lanka. The study was further extended to six different localities in three districts of the country. Mosquito collections were done in monthly intervals from 1800 to 2000 hrs using bird-baited traps. One to three-month-old domesticated female Japanese quails (*Coturnix japonica*) were used as bird baits. All traps were placed at a height of 1.5 – 3.0 m from the ground level where nesting and activities are frequent in common birds of these study sites. Collected mosquitoes were

identified into the species level using standard taxonomic keys and molecular techniques. Statistical analyzes were performed to compare the diversity, distribution, and abundance of ornithophilic mosquitoes.

Objective II: Distribution of ornithophilic mosquitoes at three different height levels, *i.e.* 1 m, 3 m, and 6 m, were assessed at four habitat types located in the Kandy district of Sri Lanka which represents secondary natural forests and forested areas in human settlements. The selected height levels represent the forest floor (1m), understory vegetation (3 m), and the canopy level (6 m). Mosquitoes were collected using bird-baited traps and collected individuals were identified using standard taxonomic keys and molecular tools (DNA Barcoding). Data were plotted and statistically analyzed to show the differences in the vertical distribution of ornithophilic mosquitoes.

Objective III: Common birds were captured using standard mist nets and minute blood samples were obtained to identify the haemoparasites in bird blood. The most common ornithophilic mosquitoes were used to detect the prevalence of haemoparasites. The total DNA from bird blood was extracted using QIAGEN®DNeasy blood and tissue kits and the total DNA of individual mosquitoes was extracted following the Livak extraction method (Livak, 1984). The extracted DNA was subjected to a nested PCR method. The positive samples were sequenced and annotated using the GenBank database (https://www.ncbi.nlm.nih.gov/) and the BLASTn tool. A Bayesian tree search was carried out in MrBays software for 10 million generations and a Median-joining network was constructed in PopART software.

Objective IV: Mosquito larvae were collected from nine study locations covering highland forests, lowland forests, and human settlements of the country. Larvae were collected from both artificial and natural breeding places of mosquitoes. Collected larvae were reared separately at standard conditions in an insectarium and emerged larvae were identified morphologically and using molecular tools. The physiochemical characteristics such as temperature, pH, dissolved oxygen, conductivity, and total dissolved solids of breeding site water were

measured and used to compare the diversity of mosquitoes and co-existing sympatric species. Using the gathered data, the preference of mosquitoes to different breeding sites, the impact of water quality, emergence rate, male-to-female ratio, and co-relation between the water amount and larvae abundance were assessed.

Results and Discussion

A total of 5893 individual mosquitoes were collected and 27 ornithophilic mosquito species that belong to eight genera (*Aedeomyia*, *Aedes*, *Anopheles*, *Armigeres*, *Coquillettidia*,*Culex*,*Mansonia*, and *Orthopodomyia*) were identified. Of these, 56% were *Culex* species, which represents 41% of the *Culex* species in the country. The most abundant species were *Culex sitiens* (36.7%), *Cx nigropunctatus* (20.7%), *Cx pseudovishnui* (18.7%), and *Cx quinquefasciatus* (13.1%), whereas the latter two were common to all climatic zones and habitats. Two active peaks of ornithophilic mosquitoes in human settlements and forest areas were identified as from 1900 to 2100 hrs and 1800 to 2100 hrs, respectively. Generalized-feeding mosquitoes were mostly reported from human settlements, while more specialized feeders were mostly reported from forested habitats. The abundance of ornithophilic mosquitoes was significantly different along the vertical axis of the vegetation [forest floor, understory vegetation, and the canopy ($p < 0.05$)], however, the diversity of the mosquito species was not significant along the vertical axis. Tree holes were identified as the most preferred breeding places for ornithophilic mosquitoes. The amount of dissolved oxygen, total dissolved solids, pH, conductivity, and temperature were affected significantly by the abundance of mosquitoes. *Aedes albopictus* and *Armigeres subalbatus* was the most frequently encountered co-existing pattern. Four species of *Haemoproteus* and five species of *Plasmodium* were identified from blood samples of seven bird species (four residents and three migratory). Red-vented Bulbul, Asian Brown Flycatcher, and India Pitta were positive for *Plasmodium* spp., while Yellow-browed Bulbul, Oriental White-eye, Brown-headed Barbet, and Indian Blue Robin were positive for *Haemoproteus* spp. Two mosquito species (*Cx nigropunctatus* and *Cx pseudovishnui*) were also positive for *Plasmodium* (3) and *Haemoproteus* (1) species. The majority (85%) of the *Plasmodium* and *Haemoproteus* sequences of this study was not linked to the well-characterized species suggesting the restricted distribution of these novel parasitic lineages in the region.

Conclusion

This study identified 27 species of mosquitoes as ornithophilic mosquitoes that feed on bird blood. The results also revealed that ornithophilic mosquitoes are adapted to feed actively during the roosting time of birds and their distribution is significantly different along the vertical axis of the vegetation. The distribution pattern of these mosquitoes revealed that more specialized feeding ornithophilic mosquitoes reside in forest habitats while more generalist feeders are abundant in human settlements. *Culex nigropunctatus* and *Cx quinquefasciatus* were the dominant species in human settlements while the forest habitats were dominated by *Cx pseudovishnui*. The amount of dissolved oxygen, total dissolved solids, pH, conductivity, and temperature were affected significantly to determine the abundance of mosquitoes. Tree holes were identified as the preferred breeding places for ornithophilic mosquitoes. Haemoparasites belonging to two genera, *Plasmodium* and *Haemoproteus*, were detected from both ornithophilic mosquitoes and birds in Sri Lanka. *Culex nigropunctatus* and *Cx pseudovishnui* mosquitoes were identified as potential vectors for transmitting avian malaria parasites among bird communities in Sri Lanka. Furthermore, this study highlighted the potential transmission risk of avian malaria parasites from migratory birds to resident birds and *vice versa* through ornithophilic mosquitoes. However, extensive field surveys are essential to comprehend the transmission cycles and the prevalence of haemoparasites in the country.

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COMPOSITION OF TERRESTRIAL PEST GASTROPODS AND THE DEGREE OF ECONOMIC DAMAGE TO AGRICULTURAL CROPS IN NUWARA ELIYA DISTRICT, SRI LANKA

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Pest gastropods are one of the major groups of agricultural pests that cause significant economic losses in agriculture in many countries. They cause frequent and heavy damage to cereals, fruits and vegetables (Kozlowski and Kozlowski, 2011). The degree of damage to some of these vegetables from pest gastropods may be as severe as 80% (Kozlowski and Kozlowski, 2011). Pest gastropods not only inflict damage on crops but they cause serious damage to ornamental plants and herbs as well (Kozlowski 2005; Kozlowski and Kozlowski, 2011; Honek and Martinkova, 2014).

An island wide survey of terrestrial gastropods carried out in Sri Lanka from 1999 to 2002 highlighted the presence of 18 species of exotic gastropods in the country (Mordan *et al*., 2003). Most of these exotic species were recorded in association with agricultural land in the wet and the intermediate zones of the country where they cause considerable damage and economic loss to a variety of crops (Kumburegama and Ranawana, 2002; Naggs *et al*., 2003).

One of the most important prerequisites in the containment and control of any pest would be the availability of precise information with regard to their biology, distribution and ecology. However, no monitoring programs or studies on any aspect of pest gastropod control, biology, ecology, distribution, or damage to crops have been carried out in Sri Lanka. Furthermore, there is a possibility that more exotic species were introduced to the island during the past decade (MOE, 2012; Ranawana, 2012). The current study aimed:

I. To document the diversity of terrestrial pest gastropods and their distribution in agricultural lands

II. To determine the seasonal variation of these terrestrial pest gastropods in agricultural fields

III. To estimate the degree of damage and economic loss caused by terrestrial pest gastropods to crops

IV. To document the invasive potential of terrestrial pest gastropods into natural and semi-natural forest habitats in the Nuwara Eliya (NE) district**.**

Methodology
Study site
Eighty agricultural lands and sixty natural and semi-natural forest patches adjacent to the agricultural lands in the NE district were sampled from 2017 to 2019.

Sampling methods
Field sampling was carried out during the night (1800 h to 0100 h), when the pest gastropods are most active. Each file was visitedduring both the rainy and non-rainy periods. Natural and semi-natural habitats were sampled during early morning (0600 h to 1000 h) and evening (1600 h to 1900 h).

Ten 1 m^2 plots were established in each agricultural location and scanned for gastropods. Each plot was sampled for 15 minutes. Species encountered in each field visited were identified and photographed. The types of crops cultivated and the pest abundance in each field were recorded. During each field visit intensity of damage to crops from these pests were evaluated by observing 10 plants per plot, per crop species. The number of damaged plants; type and extent (%) of damage were recorded.

Invasive potential of the pest gastropods was assessed by establishing ten to fifty 1m^2 plots from the edge of the natural habitat to its interior and sampling for all terrestrial gastropods. Geo coordinates, altitude relative humidity, atmospheric temperature, soil pH and daily rainfall data were recorded for each location.

Data analysis
All the statistical analysis were conducted in R studio. The Chao 2, Jacknife 2 and species based rarefaction were estimated using EstimateS 9. Temporal variations in rainy and non-rainy periods were compared using paired t test, Shannon Wiener index, Simpson dominance index, Shannon evenness index and Jaccard index.

Pearson's correlation values were calculated between measured environmental variables and species richness and abundance with 95% confidence limits. A Canonical correspondence analysis (CCA) was carried out to determine the relationship between the pest gastropod composition and measured environmental variables. Species distribution maps were prepared using the Inverse Distance Weighted (IDW) interpolation method in Arc Map 10.4.

Results and Discussion
A total of 5758 and 1325 individuals of gastropods were encountered during the rainy and non-rainy periods respectively. The abundance of the species in the rainy period was higher than the non-rainy period. Altogether 13 species belonging to seven families were identified during the rainy period while nine species belonging to five families were identified during the non-rainy period and it was significant. Almost all the species except *Milax gagates*, *Subulina octona*, *Allopeas gracile*, *Ariophanta bistrialis* and *Ratnadvipia irradians* were found in both rainy and non-rainy periods while, *Cryptozona chenui* was recorded only in the non-rainy period (Figure 1).
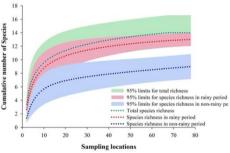

Figure 1: Species based rarefaction curves with lower and upper 95% confidence boundaries constructed for terrestrial gastropods encountered from the agricultural lands in the Nuwara Eliya district

The relative abundance and density of each species were higher during the rainy period. However, the difference was significant only for *Bradybaena similaris*, *Deroceras reticulatum*, *Lissachatina fulica*, *Mariella dussumieri* and *Macrochlamys indica*. The highest relative abundance was shown by *D. reticulatum* during the rainy period (40% relative abundance). The relative abundance of the other sampled species was less than 10% in both the non-rainy and rainy periods except for *B. similaris*, which showed a relative

abundance of 19% in the rainy period (Figure 2).

Shannon Wiener diversity and Simpson dominance indices were highest for the gastropods during the rainy period ($H^{'} = 1.60$ and $D_s = 0.70$) compared to the non-rainy period ($H^{'} = 1.45$ and $D_s = 0.69$). Evenness is highest in the non-rainy period ($S_e = 0.63$) and lowest in the rainy season ($S_e = 0.62$). Similarity of the sampled gastropods between the two seasons was 57%.
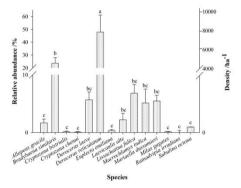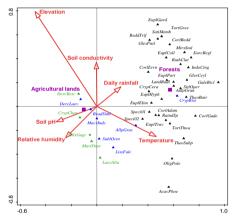

Figure 2: Relative abundance and density of terrestrial gastropods in agriculture lands in the Nuwara Eliya district (N=7083; in 1608 plots within 14,960 m² total area in agriculture lands) from July 2017 to July 2019. Simple alphabetical letters denote the significant level for each value.

Climatic factors play an important role in gastropod distribution. The distribution of the native and endemic species was highly affected by the relative humidity and daily rainfall. Yet the exotic species thrive in agricultural lands influencd mainly by elevational and atmospheric temperature. Thus, the distribution of species such as *Deroceras laeve*, *D. reticulatum*, *M. gagates* and *M. dussumieri* were mainly governed by the elevation and atmospheric temperature. Rainfall and soil pH affected the spatial arrangement of *C. chenui*, *M. indica*, *S. octona* and *Euplecta emiliana*. Relative humidity was the governing factor in the distributions of *A. gracile*, *Leavicaulis alte*, *L. fulica*, *A. bistrialis* and *R. irradians* (Figure 3).

According to the correlation analysis, daily rainfall significantly correlated with species richness while elevation, atmospheric temperature and soil conductivity significantly correlated with species abundance.

The seedling stage of leafy vegetables like leeks, cabbage and lettuce, rooted crops like carrot and chili were the most vulnerable to gastropod attacks. Apart from these, beans, beetroot, bell pepper, broccoli, Chinese cabbage, cucumber, gherkin, capsicum, knolkhol, onion, ornamentals flowers, radish, zucchini and tomato were also attacked to a lesser degree. The main terrestrial pest gastropod species that cause this damage in NE were *D. reticulatum* and *B. similaris* due to high population densities. Terrestrial pest gastropods exhibit marked preference for certain crop species and the damage was severe during the rainy period. Yet, the field damage was less than 10% in most of the locations in NE.

Figure 3: CCA of species-environmental arrangement in natural forests and agricultural lands in Nuwara Eliya district. Blue, green and black colors indicated shared species between forests and the agricultural lands, species found only in the agricultural lands and species only found in the forest habitats respectively.

Some of the exotic pest gastropods are considered as invasive species in some countries where they pose a threat to the native biodiversity. In the NE district, the agricultural fields are in close proximity to the natural forest buffers. A high number of the exotic pests rest in marginal vegetation located between natural forests and cultivated lands. Notably, while most of the core zones in the natural forests were inhabited by native gastropod species, exotic gastropod species (*L. fulica*, *D. laeve*, *A. gracile*, *S. octona* and *B.*

similaris) were present along the natural forest buffers in high numbers.

Conclusion

Environmental factors such as daily rainfall, elevation, temperature and soil conductivity affect the distribution, composition and abundance of gastropods in agricultural fields. The effects of environmental factors, as revealed by the results of this study, can be used to formulate management plans to control the occurrence and distribution of pest gastropods in the Nuwara Eliya district. It will ultimately help reduce the economic loss to the farmers and help safeguard native biodiversity.

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IMPACT OF ENVIRONMENTAL DISTURBANCES ON FIGS AND THEIR WASP POLLINATOR INTERACTION

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Vegetation of the world is dwindling and degrading significantly in an accelerated manner, mainly due to the anthropogenic activities and climate change. The changes in vegetation are adversely affecting the wellbeing of the flora, fauna and human being, and leading to critical alterations in their life patterns. Some groups of plants and animals are getting affected irreversibly than the others; especially the obligatorily mutually dependent associations.

The fig/fig-wasp mutualism is considered the quintessential example of co-evolution and co-speciation, each fig (*Ficus*: Moraceae) species interacting with a unique pollinator wasp (Chalcidoidea: Agaonidae) species, *i.e.*, basically each species of fig tree has an obligate mutualism with its pollinating wasps (Janzen, 1979). Modifications of fig-wasp species composition, changes in sex-ratios, reduction of fig/fig-wasp symbiosis and leading them to parasite/host systems due to the alterations in habitats, have been reported mainly due to habitat disturbance and forest fragmentation (Patel, 1998; Ma *et al.*, 2009; Wang *et al.*, 2015). Furthermore, in some extreme cases, extinction of pollinator fig-wasps and local extinction of the relevant fig (*Ficus*) species as a consequence have also been postulated (Mawdsley *et al.*, 1998).

Hence, the present study was conducted to investigate the effect of three different disturbance regimes (vegetation cover / tree density) on syconial features, seed production and pollinator and non-pollinator fig-wasp production of four *Ficus* species (*viz.*, *Ficus callosa*, *Ficus exasperata*, *Ficus hispida* and *Ficus racemosa*) through fig/fig-wasp pollinator mutualism, in three different sites in Kandy District, Sri Lanka with different disturbance regimes.

The specific objectives of the study were,

(I) To record the distribution of the four fig species in three selected disturbance regimes (vegetation cover) to investigate the grouping/isolation of individual trees of each fig species.

(II) To evaluate the effect of vegetation cover (disturbance) on syconial features of four species of figs and the seed production of two monoecious fig species; *Ficus callosa* and *Ficus racemosa*.

(III) To determine the species composition and abundance of pollinator and non-pollinator wasps in syconia of the four fig species in three disturbance regimes.

(IV) To determine the effect of non-pollinator wasps on fig-pollinator mutualism in the four fig species distributed in the three disturbance regimes.

(V) Based on the findings, deduce the effect of forest disturbance (effect of vegetation cover) on fig-pollinator mutualism and distribution of individual *Ficus* tree species.

Methodology

This study (site selection, fig tree survey, syconia collections and preservation) was done from January 2012 to December 2013 and enumerations were carried out up to August 2015.

Three study sites were selected from Kandy district based on their disturbance regimes, *viz*., Kandy Municipal Council area as the highly disturbed site, the park of University of Peradeniya as the moderately disturbed site and a traditional village setting in Thumpane-Hatharaliyadda area as the less disturbed site.

Two monoecious fig species (*Ficus callosa* Willd. and *Ficus racemosa* L.) and two dioecious fig species (*Ficus exasperata* Vahl and *Ficus hispida* L.f.) were selected. Fig trees within 1 km radius area (3.143 km^2) were identified and recorded.

Mature syconia were collected from the selected trees, length and diameter of them were measured, reared the syconia for wasp emergence. Syconia and fig-wasps that emerged from individual syconia were preserved separately in 80% and 70% alcohol respectively (Karunaratne, 2009).

Florets (used/galls and intact) were counted. Pollinator and non-pollinator fig-wasps were identified (Priyadarsanan, 2000), sexed and counted.

Data were analyzed using ANOVA, Mood's median test and Pearson correlation coefficient, using MINITAB Version 16.

Results and Discussion

Number of trees of all *Ficus* species (*i.e.,* the density of trees) was relatively low for all sampling sites, most of them were at their sapling stage and were not mature enough to produce syconia.

Moderately fragmented and least fragmented sites harbour the greatest number of individuals of dioecious *Ficus exasperata* and *Ficus hispida*, and monoecious *Ficus callosa* and *Ficus racemosa*. Whereas, the highly fragmented site harboured the least number of trees belong to the four species of figs.

Except *Ficus hispida* in the highly fragmented area, it was clear that disturbance has no direct influence on the production of syconia in the four species of figs studied during the study.

In both *Ficus exasperata* and *Ficus racemosa*, syconial length, syconial diameter, syconial volume and number of florets per syconia among the three sites were significantly different and mean values of all characters were recorded highest in the least disturbed site.

Species composition of the pollinator and non-pollinator fig-wasps of the four fig species in the three study sites did not vary with the degree of disturbance. Results indicated that the level of disturbance has no effect on the percent wasp production from syconia of the four species of figs collected from the three sites.

Ficus exasperata investigated during the present study revealed that the number of non-pollinator fig wasps has significantly increased with increasing disturbance and hence fig/pollinator mutualism is at a risk even though the pollinator production was not significantly different among the three sites.

In *Ficus callosa* disturbance enhances pollinator production and decreases non-pollinator production. In both *Ficus hispida* and *Ficus racemosa* pollinator production increased with disturbance.

Cauliflorous fruiting clusters of *Ficus hispida* 'male trees' that are truly geocarpic were reported during the study for the first time in Sri Lanka, and not elsewhere.

Conclusion

It was clear that the disturbance has no direct influence on the production of syconia in three species of figs studied, except in *Ficus hispida* in the highly disturbed area. Species composition of the pollinator and non-pollinator fig-wasps of the four fig species in the three study sites did not vary with the degree of disturbance. The level of disturbance has no effect on the percent wasp emergence from syconia of the four species of figs in three sites. In *Ficus exasperata,* the number of non-pollinator fig-wasps increased significantly with increasing disturbance. In *Ficus callosa,* it is apparent that the disturbance enhances the pollinator production and decreases non-pollinator production. In *Ficus hispida* and *Ficus racemosa*, pollinator production increased with disturbance. It indicates that the different fig (*Ficus*) species and their pollinator and non-pollinator fig-wasps may experience the degree of disturbance in variable, even contradictory ways. Factors that govern the reproduction in these four keystone fig species will be of value to the conservation of *Ficus* species in general, that are threatened by habitat disturbances. This study highlights the importance of reducing the habitat/forest disturbance in order to enhance pollinator abundance; and also provides pioneering information on taxonomy of Sri Lankan fig-wasps to carry out further studies on this model-system of plant/animal co-evolution.

Source of Funding

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- PHOTOCATALYTIC AND NOVEL PHOTOVOLTAIC APPLICATIONS OF $Ag/AgBr/TiO_2$ PHOTOSENSITIVE NANOCOMPOSITE
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- MILLENNIAL TO SUB-MILLENNIAL SCALE VARIABILITY OF THE INDIAN WINTER MONSOON AND ITS IMPACTS ON COASTAL ECOSYSTEMS OF SOUTHEASTERN SRI LANKA
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M.Sc. Degrees (SLQF 10)

- MORPHOLOGICAL AND MOLECULAR IDENTIFICATION OF *Colletotrichum* SPECIES ASSOCIATED WITH BANANA ANTHRACNOSE DISEASE IN THE CENTRAL PROVINCE
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- CORROSION PROTECTION OF MILD STEEL USING POLYPYRROLE AND CLOVE OIL COMPOSITE
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- CONTROLLED-RELEASE DRUG DELIVERY FORMULATION BASED ON METHOTREXATE-LOADED HALLOYSITE NANOTUBES
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- CROSSLINKED NANOHYDROGEL BASED ON CARBOXYMETHYLCELLULOSE AND CHITOSAN FOR CONTROLLED DELIVERY OF METHOTREXATE
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