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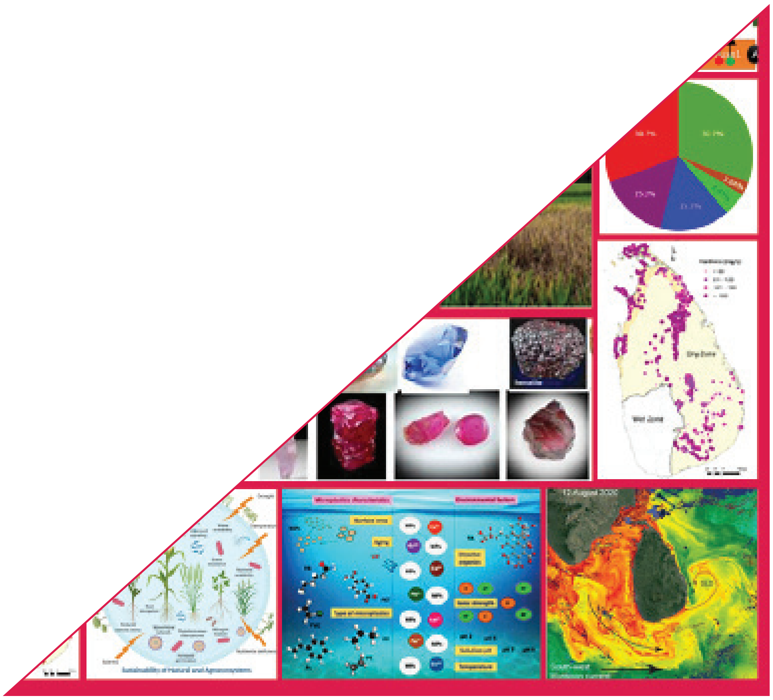

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JOURNAL OF THE
NATIONAL SCIENCE FOUNDATION
OF SRI LANKA

Volume 50 Special Issue

Special Issue on **Meeting Global Challenges: A Transdisciplinary and Transnational Approach**

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EDITORIALS

JNSF Milestones

2022 Impact Factor raised to 0.682

2021 Impact Factor 0.515

2019 Early publishing of Abstracts

2018 Online processing through Sri Lanka Journals Online (SLJOL) platform

2013 Open Access

2010 First impact factor recorded

2008 Indexed in Science Citation Index Expanded

2006 Face-lift of Journal cover page
Cited in BIOSIS Previews and Zoological Records

2005 Appointment of the International Editorial Advisory Board

1998 Title changed to Journal of the National Science Foundation

1994 Number of issues per volume increased to four from two

1972 Launch of first volume - Journal of the National Science Council

Message from the Chairman

It affords me great pleasure to write this message for the commemorative volume of the Journal of the National Science Foundation (JNSF), issued under the theme "Meeting Global Challenges: A Transdisciplinary and Transnational Approach", to mark its 50th anniversary. Founded in 1972, the JNSF, the leading national journal in science and technology in Sri Lanka, became indexed in the Clarivate Analytics Science Citation Index Expanded in 2008 (by Thomson Reuters then). In 2022, the impact factor of the JNSF reached 0.682, which shows it is on an upward trajectory. The maintenance of high editorial standards by the Editors-in-Chief and the members of the editorial board since its inception, and the high quality of the papers submitted by scientists at home and abroad, have contributed to the journal's success, which we record with deep appreciation.

Multi-disciplinarity draws upon knowledge from different disciplines while remaining within their boundaries, whereas inter-disciplinarity analyzes, synthesizes and harmonizes links between disciplines into a coordinated and coherent whole. The latter is an integrated approach to addressing questions that recognizes the limitations inherent in the compartmentalized structure of academic research. In contrast, transdisciplinarity moves beyond the bridging of divides within academia to engaging directly with the production and use of knowledge outside of the academy. In this approach, societal impact is set as a central aim of the research. The approach requires the integration of academic researchers from different disciplines with non-academic participants for co-creating new knowledge and theory so as to address complex societal challenges and global issues.

Today, despite phenomenal advances and remarkable accomplishments in science and technology (S&T), conflicts, terror, violence, unrest and disquiet are mounting in a world characterized by volatility, uncertainty, complexity and ambiguity. This world is becoming increasingly more chaotic, more insecure and more dangerous by the day. For instance, the world has to come to grips with a myriad of formidable challenges and threats such as climate change, loss of biodiversity, pandemics, natural hazards, high-tech terrorism, drug trafficking, arms trafficking, cybercrime, air pollution and marine pollution while nevertheless seizing the emerging opportunities for the benefit of humanity. In addition, with the advent of the free economy and globalization, there is unhindered flow of capital, services, goods and labour across the globe. Capital is sourced from where it is cheapest, talents drawn upon from where they are best available, goods produced where it is cheapest to make them, and products sold where markets are not constrained by national boundaries. For instance, Apple phone designed in the USA is assembled in China and made in 31 countries with over 785 suppliers.

Therefore, in a globalized environment, no country can be independent of or insulated from what is happening elsewhere; all countries have become interdependent and interconnected. This is amply evident from the current Russia-Ukraine war, which has affected not only Europe, but the whole world, in which countries are connected by innumerable supply chains and value chains that account for about 70% of international trade. Therefore, today we need to accept and recognize the oneness of humanity. Moreover, contemporary sustainable development challenges, like the global challenges referred to above, are complex, multi-faceted and multi-dimensional. Tackling them demands a transdisciplinary, transnational approach where cooperation between specialists with diverse backgrounds in both the natural and social sciences across territorial boundaries is imperative. Besides, there is growing recognition that new approaches and different types of expertise are needed to renew science. It is against this backdrop that the theme chosen by the NSF for the commemorative volume is so timely.

However, it should be stated that, while it is necessary to approach problems holistically from multiple angles, each approach must be rooted in strong disciplinary foundations. Therefore, there is still the need to continue advancing the science of each discipline in order to conduct transdisciplinary research effectively. Thus, the respective relevance and importance of monodisciplinary, multidisciplinary, interdisciplinary and transdisplinary research depends on the specific context and should be pursued and applied as appropriate.

A salient feature of the commemorative volume is that nearly half of the papers have been contributed by internationally reputed scientists of Sri Lankan origin, a significantly greater number than in the past. This progress was facilitated by the global digital platform constructed by the NSF to harness Sri Lankan expatriates for enhancing S&T and national development. We propose to invite more contributions from foreign scientists across the globe in the future by leveraging the International Editorial Board of the Journal as well as the networks of the NSF. Here, attempts will be made to strike a fair balance between papers based on monodisciplinary, interdisciplinary and transdisciplinary research. It is hoped that such interventions will enhance the value, global presence and impact factor of the Journal.

While expressing my deep appreciation of and profound gratitude to the Editorial Board, reviewers, contributors and the staff of the Journal Publication Division for their concerted, indefatigable effort, consistent support, and invaluable contribution to sustaining the JNSF in the past 50 years, I also look forward to their continued support and their commitment to propelling the Journal to even greater heights.

Prof. Ranjith Senaratne
Chairman
National Science Foundation

Message from the Director General

Disseminating scientific information propels advancement of science, while bringing more visibility to scientists and their findings. Current business of Academic Publishing has ever increasing demand and plethora of journals published by many different organizations add value to global knowledge repository. The Journal of National Science Foundation is one such ‘local gem’ in the “global mine’ of academic journals. Since its first publication volume, the journal has had steady and progressive development over the years, passing many milestones. The JNSF is an asset to the NSF as well as to the country, being the only indexed Sri Lankan journal in the Science Citation Index Expanded.

Academic publishing is a bridge connecting current research and future research. The special issue theme “Meeting global challenges: A transdisciplinary and transnational approach”, envisage future of research and its orientation with multi-sectoral approach.

It is no doubt that the JNSF has been the cradle for many Sri Lankan budding scientists, who later spotted global scientific landscape. Since 2008, the year the Journal Indexed in the Science Citation Index Expanded, tremendous increase in local and foreign submissions have been observed. In 2021, the total number of submissions increased to 514 from 68 in 2008 with gradual increase of local submissions during past few years indicating the equal demand from home and abroad.

As per statistics, Asian continent is heading the submissions compared to other global counter parts, signaling the strategic need in next few decades to attract articles from other regions.

At this golden jubilee celebrations, NSF humbly thanks the present and past Editorial Board members and the present and past members of the International Editorial Advisory Boards for their honour role played to take the journal to a greater height. Dedication and devotion made by reviewers in making a series of JNSF volumes, rich in scientifically valuable articles is appreciated. I salute all authors who trust JNSF to publish their valuable research findings and be partnered in JNSF journey. We honour you for considering JNSF as your academic publisher. The silent role played by past and present members of the Editorial Staff is unparallel in bringing the JNSF to the present status.

Scientific research has shed light and made immense contribution to control the pandemic situation. Thus, the role of academic journals needs to be more responsible; JNSF responsibility would be no difference to others. Hence, the JNSF voyage in next five decades, though starts in a dynamic socio- economic and political atmosphere, need rapid and responsive by overcoming challenges viz; reducing turn-around- time and raising the impact factor.

It is with great honour and pleasure, I extend my warm wishes to the JNSF with heartfelt congratulations, at the time the Journal celebrates its 50-year proud journey. I wish a yet another successful 50-year journey bringing credit to the country and NSF, elevating the journal to high impact status.

Dr Sepalika Sudasinghe
Director General
National Science Foundation

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DOI: http://dx.doi.org/10.4038/jnsfsr.v50i0.11311

GUEST EDITORIAL

A tribute for fifty years of dedication and teamwork

There is an increasing focus the world over on science being linked to the provision of changes necessary to find integrated, lasting and practical solutions to problems faced by the planet and its inhabitants. This underscores the importance of developing the capacity for high quality scientific research in a country.

Mandated by the National Science Council of Ceylon Act No. 9 of 1968, of the Democratic Socialist Republic of Sri Lanka, the National Science Council of Ceylon was established in 1968. The council was conferred, among other powers, to collect and disseminate information relating to scientific and technical matters, and to publish reports, periodicals and papers on matters scientific and technical. The vision and mission have prioritized the promotion of science, technology and innovation of socio-economic development of the nation.

Quality science, technology and innovation research alone does not ensure practical solutions which lead to answers for developmental problems of a country. It is extremely important for scientific information regarding insights gained through reliable and quality scientific research to reach fellow scientific peers. It is also important for this information to be communicated to the general public, the government and its management as well as the politicians to ensure their support to establish the desired changes/solutions for developmental problems.

Effective science communication is an important part of dealing with science. There are many ways in which attention to the communication aspects of science improves science itself. Good scientists are also good communicators who pay attention to both the message being communicated and the presentation aspect of a manuscript.

The National Science Council commenced the publication of Volume 1 No 1 of the Journal of the National Science Council in 1972 with two issues per year. The scope of the journal was extended to all branches of science and technology. From 1994 to date the journal has published four issues per year in March, June, September and December. In 2006 the JNSF was published with a face-lift which was much appreciated by the readers.

The year 2006 was very special for the National Science Foundation, the scientists of the country and the state of science in the country. This was due to the journal being cited in two databases of the Thompson Scientific Index. The two databases were the BIOSIS Previews and Zoological Records. Two years later in 2008 the journal was also indexed by the Science Citation Index Expanded of the Thompson Scientific Index which is currently owned by Clarivate Analytics. The journal is now indexed in the Scopus, Biological Abstracts and Chemical Abstract as well as the indexing databases TEEAL, Ulrich's, EBSCOhost, and CAB Abstracts. All this depicts the added acceptance of the journal indicating the growth of science in the country.

Currently the Journal accepts submissions under four categories. These are Research Articles, Reviews, Research Communications and Correspondence from local and overseas researchers/scientists. The Editorial Board decision to shift to online submission of manuscripts and processing them through the Sri Lanka Journals Online platform (SLJOL) saw a considerable increase in the number of submissions as well as a reduction in the time required for processing. The journal is published both online and in print form. Additional features like the ability to access the full text of the journal through the SLJOL website *http://jnsfsl.sljol.info/* free of charge and lifting the embargo period of 06 months and becoming open access since 2013, has increased the circulation of the journal locally as well as overseas. The Impact Factor of the journal has shown an increasing trend and was 0.682 for 2022.

This special issue of the journal with the theme "Meeting Global Challenges: a Transdisciplinary and Transnational Approach" celebrates and appreciates fifty years of continuous publication. The JNSF is one of the best examples of teamwork in science in the country. It is with grateful thanks that all team members over the years are remembered for their contributions to this success story.

Often the persons at the forefront of an activity are appreciated and admired more than the persons who work hard behind the scenes. It is they who ensure that the final product is a reality. For this they deserve credit and grateful thanks. The past and present dedicated staff of the Journal Publication Division who have shouldered their daily responsibilities regarding the publication of the journal has always been the strength behind the journal.

Foremost of the JNSF team who deserve credit are the research scientists and technologists whose untiring efforts have produced meaningful research findings which they have communicated through quality manuscripts submitted for publication in the journal. Many of the insights contained in these manuscripts have contributed to science in varying degrees and also provided answers to developmental problems of the country. The authors of these manuscripts are role models who must be followed by the young scientists of the country who have the responsibility of continuing the good practice submitting quality manuscripts for the future issues of the journal.

The Editorial Boards have been guided by the invaluable inputs of the reviewers of the manuscripts who have helped maintain the quality of the publication at a high level.

The team also includes all members of the Editorial Boards who have played key roles in the advances achieved by the journal. They have guided and given the necessary leadership to the journal. The past and present Chairman/Chairpersons and the members of the Board of Management of the National Science Foundation are thanked for their advice and guidance.

This special issue is a grateful tribute to all those who have contributed to the success of the Journal. I have no doubt that the journal will in the future achieve much more and continue to fulfil the aspirations of the scientific community of the country.

Prof. Nalini Ratnasiri
Emeritus Professor
(Editor-in-Chief/JNSF 2005-2019)

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EDITORIAL

A journey of fifty years

I consider it a privilege to be the editor-in-chief of the Journal of the National Science Foundation during the year that it is celebrating its 50th year of publication.

Prof. G. C. N. Jayasuriya was the chairman of the editorial board of the first issue of the Journal which was published on the 27th of August 1972.The Journal has been published regularly since then providing Sri Lankan scientists with a quality platform for sharing their research results with the broader scientific community. The increasing number of papers received and published from other countries in the region in the recent years, reflects the growing acceptance of the Journal internationally.

This celebratory special issue consists of invited articles from eminent Sri Lankan scientists, working in Sri Lanka and abroad. Neither the list of scientists invited nor the fields of science in which Sri Lankans have excelled as represented by the articles in this issue, is comprehensive. Nevertheless, the articles cover a broad range of subject matter, and provide a fascinating view of different areas of science and its applications, to address many issues of current importance to society.

The authors were left free to decide on the format of their articles, and as befitting the occasion, most have been written as reviews, while some which also include substantial elements of a review, have been formatted as research articles. I thank all the authors for their impressive contributions.

I am particularly happy that my predecessor, Prof. Nalini Ratnasiri, has provided a guest editorial for this issue. I thank the current editorial board for providing their expertise and editorial guidance towards maintaining and improving the standard and the standing of the journal. I also thank Prof. Ranjit Senaratne, chairperson of the NSF, for his keen involvement and support to bring out this issue.

It is a pleasure to acknowledge the smartness, capability and commitment of the staff of the editorial office of the JNSF, which has contributed in no small measure to the successful journey of the Journal over the years.

Ajit Abeysekera

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REVIEW

New paradigm for inland fisheries development in Sri Lanka: a transdisciplinary approach for addressing food and nutritional security

US Amarasinghe[1], KBC Pushpalatha[2] and WMHK Wijenayake[3]
[1] *Department of Zoology and Environmental Management, Faculty of Science, University of Kelaniya, Kelaniya, Sri Lanka.*
[2] *National Aquaculture Development Authority of Sri Lanka, Battaramulla, Sri Lanka.*
[3] *Department of Aquaculture and Fisheries, Faculty of Science, Wayamba University of Sri Lanka, Makandura, Gonawila, Sri Lanka.*

Summary: Globally, inland fisheries make a significant contribution to sustainable development across dimensions of food security, livelihoods, economic value and biodiversity. Nevertheless, aquaculture is generally considered to be environmentally unfriendly. As such, environmentally friendly approaches of increasing fish production such as fisheries enhancement receive increasing attention. Culture-based fisheries (CBF) are seen as a way forward for inland fisheries development in most Asian countries. Due to the extensive availability, reservoirs of Sri Lanka, which have been constructed in the past for irrigation, can secondarily be utilized for inland fisheries development. The reservoir fishery of Sri Lanka has been a relatively recent development during the second half of last century after the introduction of exotic cichlid species, *Oreochromis mossambicus* and *O. niloticus*. The trends in inland fish production until the 1990s suggested that under the socio-economic milieu that prevailed, for the management of reservoir fisheries, state sponsored monitoring procedures or centralized management systems was essential. Due to the concerted efforts to develop CBF in Sri Lankan reservoirs after 2000, through regular stocking of fingerlings of tilapia, Chinese carps and Indian carps, a significant development in the inland fisheries sector has been achieved. Recent attempts at stocking *Macrobrachium rosenbergii* postlarvae in inland reservoirs as part of CBF contributed to significant elevation of rural income. Also, 'human capital' in the rural institutions (i.e., famer organizations and fisheries societies) has a high potential to be mobilised for CBF development. Introduction of co-management strategies for CBF is useful for its sustainability. CBF development in Sri Lankan reservoirs should essentially be a transdisciplinary approach, which would take into account the biological productivity favouring growth of stocked fish, institutional linkages for supporting different stages of the strategy, social attitudes for adopting CBF within the realm of participatory development, and market forces driving the entire process.

Keywords: Biological sustainability, blue revolution, inland fisheries enhancement, socioeconomics, tropical reservoirs.

INTRODUCTION

As many fisheries and aquaculture development strategies aim at ensuring biological sustainability and economic efficiency of fisheries, their potential contribution to improve food and nutritional security is not fully appreciated (Béné *et al*., 2015). Furthermore, inland fisheries are crucial for many socially, economically and nutritionally vulnerable groups of people around the world (Welcomme, 2001). Nevertheless, a complete understanding of the magnitude of contribution of inland fisheries to food security is often not fully recognized, mainly due to the reason that greater interest is vested in marine fisheries and ocean management (Funge-Smith & Bennett, 2019). There is widespread expert opinion that much of the catch from inland fisheries is unrecorded, bringing about underestimates of inland fishery catches (FAO, 2020). Nevertheless, there is a

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* Corresponding author (zoousa@kln.ac.lk; https://orcid.org/0000-0002-0465-6950)

significant contribution of inland fisheries to sustainable development across dimensions of food security, livelihoods, economic value and biodiversity (Funge-Smith & Bennett, 2019).

Intensification of aquaculture practices has led to use of artificial feeds, prophylactics for disease prevention, drugs of varying sorts for disease control, growth promoters and other ways and means of increasing yields, essentially reflecting comparable developments in land–based agriculture and animal husbandry. This paradigm shift was termed as "blue revolution", which is analogous to "green revolution" to intensity agricultural food production (Cunningham *et al*., 2004). Green revolution and blue revolution are initiatives that have been adopted to increase food production to meet the demand of ever-increasing global human population. Nevertheless, adoption of environmentally unfriendly approaches in aquaculture such as increased use of antibiotics, eutrophication, consumptive uses of water etc. made "blue revolution" far from green (Costa-Pierce, 2002). As such, environmentally friendly approaches of increasing fish production such as fisheries enhancement (Lorenzen *et al*., 2001; De Silva, 2003; Taylor *et al*., 2017) receive increasing attention.

The significant growth in aquaculture in recent times has also been subjected to a higher degree of close monitoring by the public, particularly in the wake of the establishment of the convention on biological diversity (CBD, 2018). There has been no generally accepted notion that all major aquaculture development activities should be sustainable (De Silva & Davy, 2010). Overall, sustainability of intensive aquaculture is often questioned on many grounds (Naylor *et al*., 2001; De Silva *et al*., 2006) but the growth of the sector has to be considered in the context of food security and nutrition (Committee on World Food Security, 2014). Béné *et al.* (2016) have shown that the influences of major drivers (decentralization, climate change, demographic transition) on the contribution of aquaculture for poverty alleviation are still insufficiently documented and therefore poorly understood. Recently, inland fisheries enhancements, such as culture-based fisheries (CBF), have been increasingly adopted and seen as a way forward for inland fisheries development in most Asian countries (De Silva & Funge-Smith, 2005). As strategies for enhancement of inland fisheries are essentially multi faceted paradigms, this synthesis is aimed at providing a comprehensive review of inland fisheries in Sri Lanka, within the purview of transdisciplinary approaches for their sustainability.

Reservoirs of Sri Lanka and their fisheries

Sri Lanka is endowed with large number (>12,000) of irrigation reservoirs (Figure 1). In Sri Lanka, reservoir construction and use have been an integral part of ancient civilization, with some major reservoirs being more than 2,000 years old. Most of these reservoirs are concentrated in the dry zone receiving 125 – 190 cm annual rainfall. The total reservoir extent of the country is around 206,000 ha (Jayasinghe & Amarasinghe, 2018). On average, size of these reservoirs ranges from a few hectares to 7,000 hectares. Depending on their hydrological regimes these reservoirs are broadly categorized into seasonal and perennial reservoirs. These reservoirs with a few exceptions (i.e., recently constructed hill country hydroelectric reservoirs, some drinking water reservoirs and a few reservoirs of aesthetic value) are irrigation reservoirs and are very diverse in age, hydrology and catchment features (Amarasinghe *et al*., 2002). Perennial reservoirs of Sri Lanka (around 200,000 ha in extent) are divided into three broad categories: major (>800 ha), medium (200 – 800 ha) and minor (< 200 ha) based on the water spread at full supply level. These irrigation reservoirs have been secondarily used for the development of inland fisheries.

Historical evidence indicates that inland fish were exploited from reservoirs and irrigation canals during the time immemorial (Siriweera, 1986; 1994). Inland fisheries have been a significant source of food since pre-historic times. Hooks, harpoons and fish remains were found in many archaeological sites, and many early river-based civilizations. In Sri Lanka, some historical evidence is found about the fisheries in inland waters of the country (Amarasinghe, 2018). Perimiyankulam rock inscription made during the latter part of the first century refers to the revenue from fish caught in the channels of two reservoirs were used to meet expenses to spread deer skins in meditation halls of Buddhist monks (Paranavithana, 1958), indicating that inland fishing was an important economic activity during that period.

However, prior to 1950's, fish yield in Sri Lankan reservoirs was reported to be very low (Fernando, 1984, 2000). The reason for these low yields was said to be due to the inability of indigenous riverine fish species to sustain high population densities in the lacustrine habitats of reservoirs (Fernando & Holčik, 1991). Development of inland fisheries in Sri Lanka commenced with the introduction of exotic tilapia, *Oreochromis mossambicus*, in 1952 and the trend of inland fisheries in perennial reservoirs of Sri Lanka following this introduction is well documented (Indrasena, 1965; Fernando & Indrasena, 1969; Mendis, 1977; Fernando & De Silva, 1980; De Silva, 1983, 1988; Amarasinghe, 1992, 1998; Fernando, 1993, 2000; Amarasinghe & Weerakoon, 2009).

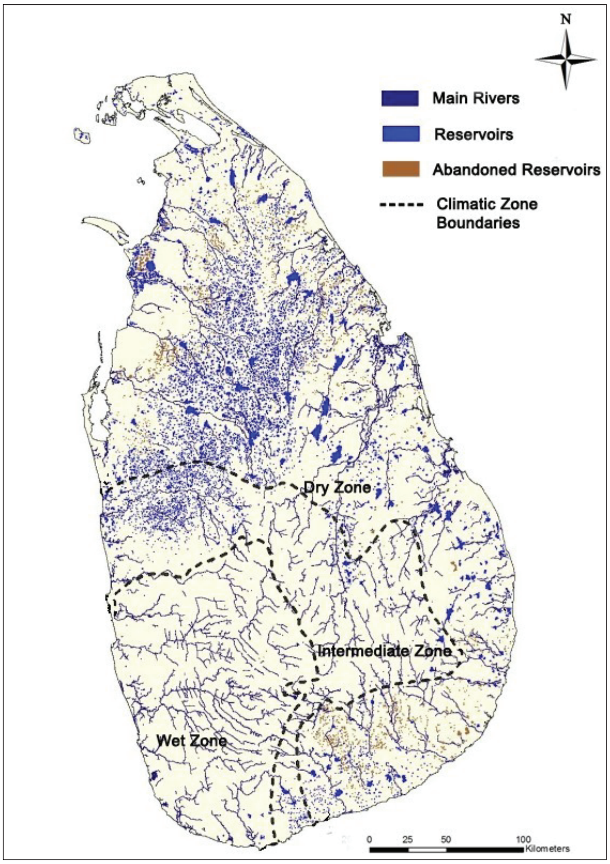

Figure 1: Distribution of reservoirs in Sri Lanka (adopted from Jayasinghe & Amarasinghe, 2018)

The impact of introduction of *O. mossambicus* into Sri Lankan reservoirs is best exemplified by the dramatic increase in fish production in Parakrama Samudra, a major perennial reservoir from <10 kg ha^{-1} yr^{-1} prior to its introduction in 1952 to 180 kg $ha^{-1}yr^{-1}$ subsequently in 1967 (Fernando, 1984). According to De Silva (1988), *O. mossambicus* became the most dominant species in the fishery of inland perennial reservoirs contributing over 70 percent of the total landings. Various reasons have been suggested to be attributed to the success of this species in Sri Lanka. Among them are breeding success in the intensive littoral zones of reservoirs (Fernando & Indrasena, 1969), ability to digest blue green algae dominant in Sri Lankan reservoirs (Costa & Abeysiri, 1978), lack of lacustrine species in the indigenous fauna capable of colonizing the reservoir habitats (Fernando & Holčik, 1991), and ability of *O. mossambicus* to change its dietary habits from season to season depending on the availability (Maitipe & De Silva, 1985). In 1975, another exotic cichlid species, Nile tilapia, *Oreochromis niloticus* was introduced to Sri Lankan freshwaters and by 1980s, it became the dominant species of the inland fisheries in perennial reservoirs following heavy stocking (Chandrasoma, 1986; Amarasinghe, 1997; Amarasinghe & De Silva, 1992a). In recent years, exotic carps, particularly catla (*Catla catla*), rohu (*Labeo rohita*) and mrigal (*Cirrhinus mrigala*) also have been contributing significantly to fish production of perennial reservoirs of Sri Lanka. Until late 1990s, the inland fisheries of Sri Lanka had essentially been capture fisheries in perennial reservoirs.

Inland fisheries production trends from 1978 to 1999

During 1978-1989 period, there were several strategies adopted by the government of Sri Lanka to develop the inland fisheries in the country. They included providing fishers with fibreglass canoes and gillnets under a subsidy scheme. As a result, fishing effort in reservoirs increased considerably (De Silva, 1988). At the same time, the fisheries authorities have also imposed fisheries regulations to control fishing effort and size of the fish (especially exotic cichlids) landed. Use of mechanised boats and any kind of shore seine nets are forbidden in perennial reservoirs and the minimum permissible mesh size for the gillnet fishery is 8.5 cm. However, gillnets of mesh sizes smaller than minimum permissible size (<8.4 cm) and beach seine, which were also illegal were operated in some reservoirs sporadically (Amarasinghe & De Silva, 1992b). Also, some fishers increased efficiency of gillnetting by beating water with wooden poles or weighted ropes to drive fishes towards gillnets (Amarasinghe & Pitcher, 1986; Amarasinghe & De Silva, 1992b).

During the early 1980s, fishers in inland reservoirs became members of the fisheries cooperative societies (FCS) because only the active members of FCS were eligible to receive boats and gillnets under the state-sponsored subsidy scheme. From the point of view of reservoir fisheries management, this trend was conducive for fishers to arrive at collective agreements regarding resource exploitation such as complete curtailing of beach seining and increasing the minimum mesh size of gillnets upto 10.2 cm. This resulted in a considerable increase in inland fish production in the country with a reported peak of 39,300 tonnes in 1989 (Amarasinghe &

De Silva, 1999). However, in 1989, the government has made a politically inspired policy decision to discontinue state patronage for inland fisheries and aquaculture development. As a result of this abrupt policy decision, during 1990-1994 period, inland fish production declined markedly (Amarasinghe & De Silva, 1999). This was due to interruption of government funding for monitoring and stocking programmes. In the absence of monitoring by fishery officers, fishers began using smaller meshed (<7.5 cm) gillnets that resulted in "growth overfishing" of the cichlid species, which were dominant in the fishery. However, inland fisheries tended to recover since the state restored its support in the mid-1990s. Trends in Sri Lanka's inland fish production from 1978-1999 are shown in Figure 2.

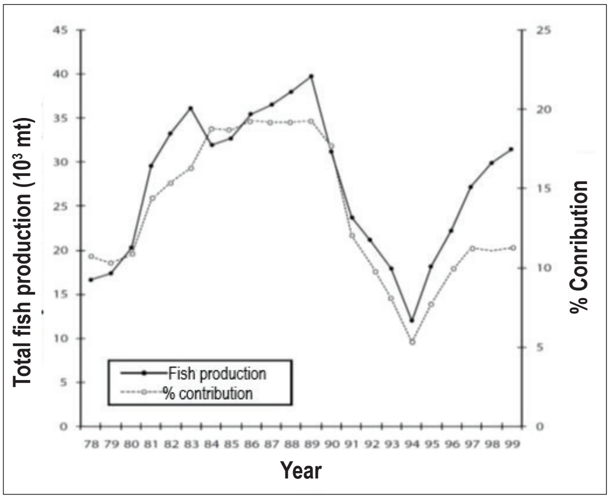

Figure 2: Trends in the inland fish production in Sri Lanka during 1978–1999 (adopted from Amarasinghe & Weerakoon, 2009). Percent contribution of the inland fishery to the total fish production is also indicated here.

These trends in inland fish production until mid-1990s suggest that under the socio-economic milieu that prevailed, for the management of reservoir fisheries, state sponsored monitoring procedures or central*is*ed management systems was essential. In two reservoirs namely Maduru Oya and Muthukandiya reservoirs, where fishing communities were actively involved in making decisions for management of the fisheries, even in the absence of centralized management system, overexploitation of fish stocked was not evident (Amarasinghe & De Silva, 1999). In both reservoirs, fishers arrived at collective decisions on fisheries management and environmental protection through the FCS. This indicated that co-management procedure in which the central*is*ed administration authority (i.e., Ministry of Fisheries) and fishing community share the responsibilities of making decisions for fishery resource management (Berkes, 1994; Pomeroy, 1995; Sen & Raajaer-Nielsen, 1996) had been useful for preventing over-exploitation of fishery resources even during the period of absence of state-sponsored monitoring procedures from 1990 to 1994 (Amarasinghe & De Silva, 1999). A similar community-based management system has also been in operation in Senanayaka Samudra (7,680 ha), the largest reservoir in Sri Lanka in terms of water spread area, where fishing trips are also regulated by the fisheries co-operative society (Kulatilake *et al*., 2010).

Inland fisheries production trends during 2000-2020

Realising the national need for sustainable development of inland fisheries in Sri Lanka, Deakin University, Victoria, Australia, University of Kelaniya, Sri Lanka and National Aquaculture Development Authority of Sri Lanka (NAQDA) developed a collaborative research project under the auspices of Australian Centre for International Agricultural Research (ACIAR) for scientific management of reservoir fisheries of Sri Lanka. Also, there was a subsequent project named as "Aquatic Resources Development and Quality Improvement Project" (ARDQIP) for the development of inland fisheries in the country, which was funded by the Asian Development Bank. The scientific inputs from both these projects have been responsible for spectacular increase

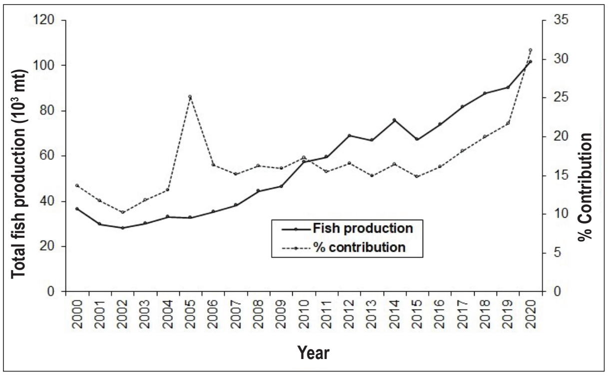

Figure 3: Trends in the inland fish production in Sri Lanka during 2000–2020 (source: NAQDA). Percent contribution of the inland fishery to the total fish production is also indicated here. Note: Increased percentage contribution of inland fishery to the total fish production in 2005 was due to drastic decline of marine fishing fleet causing decreased fish production due to tsunami on 26 December 2004.

of inland fish production in the country (Figure 3). In the ensuing section, strategies that have been adopted so far for achieving success up to now are reviewed, and the way forward for sustainability of the inland fishery of Sri Lanka is emphasized.

Culture-based fisheries

The major fisheries enhancement strategy for dramatic increase of country's inland fish production after 2001 was development of culture-based fisheries in reservoirs. In the wide array of fisheries enhancement strategies, the common feature is human intervention in the life cycle of the aquatic organism that is used for fisheries enhancement (Lorenzen *et al*., 2001), and 'culture-based fisheries' and 'capture-based aquaculture' are two major terminologies in this context. In culture-based fisheries, artificially propagated seed stocks are released into water bodies, which are not primarily and traditionally used for fish production, and recaptured upon reaching a desirable size (FAO, 1997; De Silva, 2003). As such, this is essentially 'aquaculture-driven.' In contrast, 'capture-based aquaculture,' which involves capture of aquatic organisms from the wild and rearing them in various aquaculture systems such as cages (Ottolenghi *et al*., 2004), is "fishery-driven."

As culture-based fisheries (CBF) are aimed at stocking of different species which are able to optimally utilise available food niches, they often share features of polyculture (Bardach *et al*., 1972). As a rule, culture-based fisheries involve ownership (De Silva, 2003) either singly, as in the case of Vietnam where farmers lease out small reservoirs (Nguyen *et al*., 2001), or collectively, in the form of a cooperative, such as the case in oxbow lakes in Bangladesh (Middendrop & Balarin, 1999).

Culture-based fisheries in small village reservoirs

The small village reservoirs are highly productive due to the cattle manure accumulated in the draw-down areas from cattle grazing during dry season and residues of terrestrial vegetation. Nationally, CBF development in irrigation reservoirs is of particular importance due to the fact that Sri Lanka has a long tradition of irrigation water management in reservoirs dating back to the first century AD. Small puddles in most seasonal reservoirs do not dry up completely so that they can harbour some of the indigenous carnivorous fish species with accessory respiratory organs such as snakehead (*Channa* spp.), climbing perch (*Anabas testudineus*) and catfishes (*Macrones* spp.). These indigenous species, although harvested in some reservoirs, do not produce high yields. The potential of small village reservoirs for the development of CBF was first pointed out by Mendis (1965). Accordingly, attempts were made in 1960s to utili*s*e these seasonal reservoirs for the development of culture-based fisheries (Indrasena, 1965). Indrasena (1965) reported that in 1960s, some seasonal reservoirs were stocked with *O. mossambicus* fingerlings of 7.5–10.0 cm and during 8–9 months of water retention period, they grew upto 25–30 cm in size. Fernando & Ellepola (1969) reported that in February-March 1963, eight small reservoirs in the north-central province of Sri Lanka were stocked with *Chanos chanos* and *O. mossambicus*, which were harvested in September 1963. They also reported their observations in Dalukanawewa during fishing season in July-August 1964. Mendis (1977) has estimated the culture-based fisheries production potential of the small village reservoirs as 13,000 tonnes on the basis of mean fish yield of 330 kg ha^{-1} yr^{-1}. Based on the recommendations by Rosenthal (1979) and Oglesby (1981), a programme has been implemented to formulate a suitable strategy for the development of culture-based fisheries in non-perennial reservoirs of Sri Lanka (Thayaparan, 1982).

In Sri Lankan reservoirs, irrigation of agricultural lands is essentially based on pre-planned schedules and water is not freely available on demand to the farmers. CBF can also be incorporated as a community-based activity during the planning stage of an irrigation schedule for the two cultivation seasons in a calendar year in the command areas of each reservoir. These community-based water management strategies and CBF in village reservoirs are essentially facilitated by the Department of Agrarian Development and the NAQDA respectively, under the legal provisions of Agrarian Development Act No. 46 of 2000.

As the culture period in seasonal reservoirs is 7-9 months, the species suitable for stocking in these reservoirs should reach the marketable size in 6-8 months. These species should also be able to utili*s*e the natural food resources available in the reservoirs. In Sri Lankan indigenous fish fauna, such species are not available. Exotic tilapias are not desirable because they tend to mature early in life in small water bodies. As such, culture-based fisheries in seasonal reservoirs exclusively rely on common carp, Chinese carps and Indian major carps. These species have been artificially spawned successfully in Sri Lanka (Weerakoon, 1979; Balasuriya *et al*., 1983; De Silva, 1988).

Hormone induced captive breeding of major carps in government-owned hatcheries and fingerlings rearing in mini-nurseries maintained by rural agricultural farmers are the sources of seed material for stocking in the seasonal reservoirs. These species utili*s*e the natural food resources available in these reservoirs. The efforts to develop CBF in non-perennial reservoirs in the dry zone of Sri Lanka in the 1980s (Thayaparan, 1982; Chandrasoma & Kumarasiri, 1986) were, however, not successful due to lack of a proper selection method for the identification of suitable reservoirs. Recent efforts for the CBF development in seasonal reservoirs have taken a momentum (Amarasinghe & Nguyen, 2010). A transdisciplinary approach having both resource component and social component that makes use of the resource component made it possible to achieve a considerable success in the CBF efforts in small village reservoirs of Sri Lanka.

Culture-based fisheries in minor perennial reservoirs

As fingerlings are required for stocking in non-perennial reservoirs only after the peak rainy period in November-January in the dry zone of Sri Lanka, fingerlings produced during other periods can be used for CBF development in other types of inland water bodied such as minor perennial reservoirs. In 2003, NAQDA initiated through ARDQIP, a pilot-scale programme to introduce CBF in minor perennial reservoirs (<200 ha), where only subsistence level fisheries existed. Pushpalatha and Chandrasoma (2009) reported the results of this CBF initiative in 15 minor perennial reservoirs. In these reservoirs, community-based organisations (CBOs) were formed and members were given training on basic aspects of entrepreneurship planning such as leadership, simple accounting, book keeping etc., together with aspects of community-based management of CBF. The stocking density in CBF in minor perennial reservoirs ranged from 146 fingerlings/ha to 2780 fingerlings/ha. Unlike in non-perennial reservoirs, where harvesting is done at once during the dry season, CBF harvesting in minor perennial reservoirs is a year-round activity using gillnets of mesh sizes ranging from 8.5 to 20 cm. Before introduction of CBF in minor perennial reservoirs, mean annual fish yield was reported to be 57.3 kg ha^{-1}, with *Oreochromis niloticus* being the most abundant species forming over 80% of the landings. However, after introduction of CBF, annual fish yield increased up to 208 kg ha^{-1} and *C. catla*, *L. rohita* and *C. carpio* formed major proportions of the landings. The contribution of *O. niloticus* to the landings reduced to 47.4% (range 19.7–66.5 %) (Pushpalatha & Chandrasoma, 2009; Amarasinghe, 2010). Pushpalatha *et al.* (2020) also reported that in 3 medium and 2 large reservoirs, after introduction of CBF, fish species composition in the landings changed with the occurrence of stocked species in the landings, resulting remarkably elevated fishers' income ranging from 194% to 2187%. According to the analysis of Pushpalatha *et al.* (2020), economic gains of fishing communities were due to socioeconomic characteristics having improved leadership qualities and empowerment fishers for management decision-making, which were achieved through participatory development approaches of NAQDA. However, CBF production in these reservoirs was found to be further increased through more efficient management of inputs such as increased number of fishers, increased mean number of fishing days per year and increased stocking density (Pushpalatha *et al*., 2021).

Influencing strategies for CBF development

After the re-establishment of state patronage for inland fisheries and aquaculture development in the country in 1994, the government has taken several steps to revive inland fishery. Some of the major steps taken were establishment of NAQDA and rehabilitation of state-owned fish hatcheries which were responsible prior to 1990, for induced breeding and fingerling rearing of Chinese and Indian major carps, and stocking of inland reservoirs. As small village reservoirs are managed by the rural institutions called farmer organisations (FOs), which have legal power under the Agrarian Development Act, these rural institutions were chosen to implement influencing strategies for CBF development. Meetings of FOs were held before the agricultural cultivation period ("*Kanna Meeting*"), under the supervision of Agrarian Research and Production Assistants (ARPAs) employed by the Department of Agrarian Development. These meetings provided an opportunity to discuss and place before the FOs about the possible nutritional and financial benefits that would be generated from CBF in their reservoir if they were to embark on CBF. This approach was further developed by NAQDA and the aquaculture extension officers of NAQDA, closely worked with the members of FOs in each village reservoir to develop an "entrepreneurship plan" for CBF development.

Transdisciplinary approaches for CBF development in non-perennial reservoirs

Selection of suitable village reservoirs for CBF development is needed for its sustainability in the country. Carrying out extensive surveys for selecting reservoirs is prohibitive due to limitations in terms of manpower and funding. However, 'human capital' in the rural institutions (i.e., FOs) has a high potential to be mobilised for CBF development. As such, for defining a better practice model, both biological aspects related to productivity of water bodies and socioeconomic aspects of rural communities should be considered (De Silva *et al*., 2006).

Biological and technological aspects

Carlson's trophic state index (TSI), a diagnostic approach that can be used to monitor status of lentic waters (Carlson, 1977), was employed in non-perennial reservoirs (Jayasinghe *et al*., 2005). They determined TSI (SD), TSI (Chl) and TSI (TP) according to the following definitions.

TSI (SD) = 60 – 14.41 ln Secchi depth (metres)
TSI (Chl) = 9.81 ln Chlorophyll-a (mg/m^3) + 30.6
TSI (TP) = 14.42 ln Total Phosphorus (mg/m^3) + 4.15

Jayasinghe *et al.* (2005) further showed that CBF yields were positively correlated to TSI (Chl). In non-perennial reservoirs with similar trophic characteristics, CBF yield could be predicted from shoreline/reservoir area ratio (Jayasinghe *et al*., 2006). In rural villages of dry zone of Sri Lanka, buffalo and cattle keeping is one of the economic activities. Nutrient enrichment in village reservoirs through cow dung also positively influences the CBF yields (Jayasinghe & Amarasinghe, 2007; Jayasinghe *et al*., 2018).

In village reservoirs, which do not completely dry up during the dry season, carnivorous fish species such as *Channa striata*, *Glossogobius giuris* are drawn from the associated river basins. Hence, stocked fish fingerlings are vulnerable to predation by such piscivores, resulting in low returns from the CBF efforts (Wijenayake *et al*., 2005). As suggested by De Silva (1988), this could be prevented by stocking large-sized (>10 cm) fingerlings as predator-prey relationship is usually size-dependent. However, cost-effectiveness of stocking large fingerlings in reservoirs has not yet been evaluated. Also, our experience is that when freshwater prawn (*Macrobrachium rosenbergii*) post-larvae are stocked, night stocking (after 19:00 hr) ensures higher survival. This might be due to the reason that prawn post-larvae can move to the bottom of the reservoir so that they can avoid predation by fish species such as *Rasbora daniconius* and *R. caverii* which are essentially visual feeders in the water column during twilight periods of the day (De Silva *et al*., 1996; Weliange *et al*., 2006).

Species combination of stocked fingerlings is also an important aspect in CBF because optimal utilisation of food resources in the water body is a key to achieve optimal harvests from polyculture systems (Bardach *et al*., 1972). Although not scientifically confirmed, the species combination that is commonly used (30% common carp and mrigal, 30% bighead carp/catla; 30% rohu and 10% Nile tilapia/freshwater prawn (Wijenayake *et al*., 2005, 2007), appeared to be reasonable because CBF harvests based on this species combination were generally high.

In non-perennial reservoirs, the relationship between stocking density (SD) and CBF yield showed a second order curve (Wijenayake *et al*., 2005), conforming to those that were reported from Bangladesh (Hasan & Middendrop, 1998) and India (Sugunan & Katiha, 2004). Accordingly, the optimal stocking density was determined to be 3,500 fingerlings per ha (Wijenayake *et al*., 2005). Here, it must be noted that effective area for CBF planning was considered as 50% of the area at full supply level (FSL) because extents of non-perennial reservoirs at FSL during the rainy season gradually shrink to almost zero during the dry season.

Socioeconomic aspects

As mentioned above, the community meeting of FOs held at the beginning of the cultivation season, the *Kanna meeting*, is meant for planning agricultural activities through collective decisions. At these meetings, FOs also make decisions whether CBF activities should be incorporated in the agriculture-related economic activities. As these community-based initiatives are backed by the legal provisions of Agrarian Development Act of 2000, in most instances, FOs establish Aquaculture Management Committees (AqMCs), which are responsible for stocking fish fingerings, guarding the stocked fish, harvesting and marketing. The members of AqMCs arrive at agreements with FOs on sharing of CBF profit between the AqMC and FO. In many instances, a levy of about 5 % of the net profit is paid to FOs. The group size of AqMC generally varied between 5 and 20. There are rare instances where the entire FO acts as the AqMC and as a result, all members of FO take part in the CBF activities. After payment of the levy to the FO, the profit is equally shared by the members of AqMC. Kularatne *et al.* (2009) have shown that communities with small AqMCs with satisfactory participation in group activities and those belong to the same caste expressed high 'willingness to pay' for CBF activities.

Also, socioeconomic homogeneity with regard to kinship and political ideology of the farming communities as well as education level and good leadership qualities of group members in AqMCs had a positive influence on the attitudes towards adoption of CBF in village reservoirs (Kularatne *et al*., 2009). Accordingly, socioeconomic characteristics in rural agricultural communities are important aspects to be considered for selecting suitable reservoirs for CBF development. Wijenayake *et al.* (2021) presented a simplified scheme of decision making to select suitable reservoirs for CBF development taking into account both technical and socioeconomic aspects (Figure 4).

Multi-criteria decision making

Wijenayake *et al.* (2015) employed a multi-criteria decision-making procedure known as Analytic Hierarchical Process (AHP; Saaty, 1977). In this approach, criteria that are important for CBF development in village reservoirs such as water quality parameters, catchment land-use characteristics and socioeconomic factors, under which there were several sub-criteria with varying relative importance were considered. The AHP approach equated sets of heterogeneous criteria into a common denominator, and as such, it was a powerful decision-making tool for selection of reservoirs for CBF

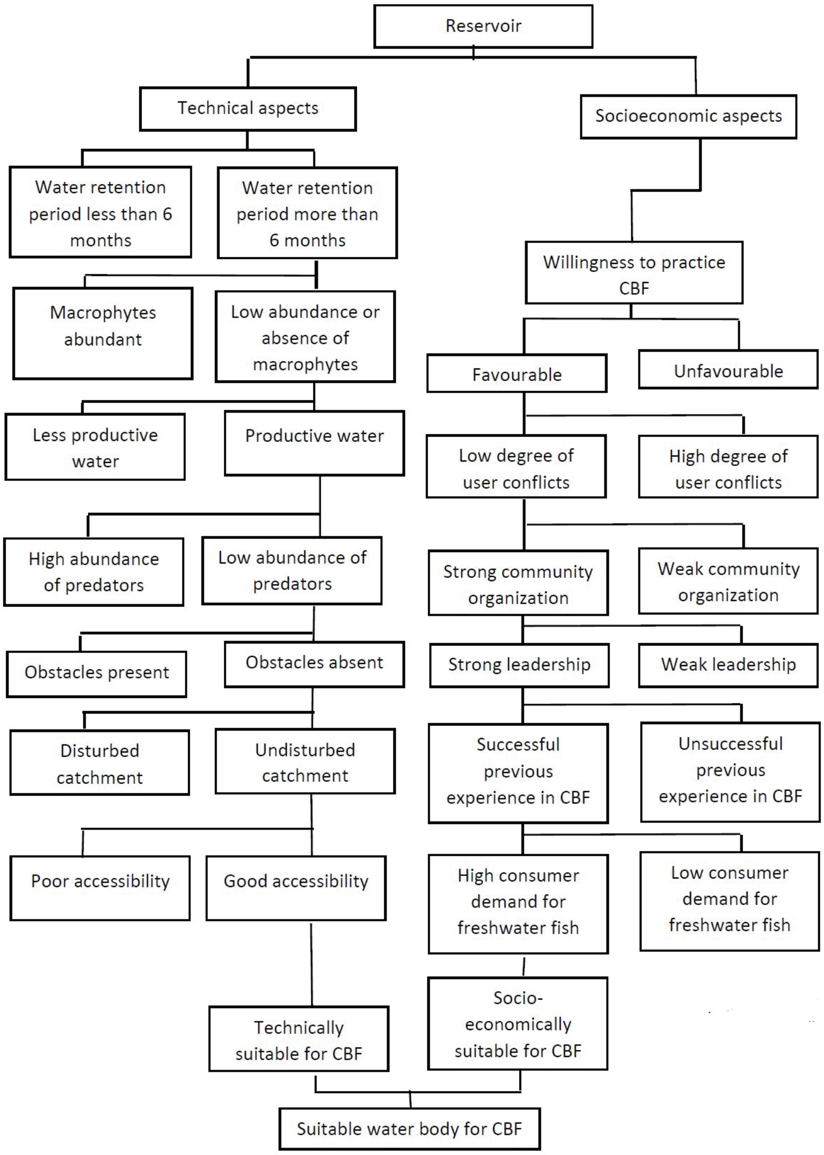

Figure 4: Technical and socioeconomic feasibility to select suitable village reservoir for CBF development (adopted from Wijenayake *et al*., 2021).

based on their ranking through relative weighting of multiple criteria.

Science into practice for CBF development

During 2004-2007, the Asian Development Bank funded ARDQIP has taken many initiatives for the CBF development in Sri Lanka. The initiatives of ARDQIP included infrastructure development in state-owned fish breeding centres (i.e., aquaculture development centres; AqDCs), capacity building through training field officers in the inland fisheries and aquaculture sector, introduction of an effective strategy for social mobilization for adoption of CBF in reservoirs (i.e., preparation of entrepreneurship plan in each reservoir through community participation), setting-up of a procedure for more accurate recording of fisheries and aquaculture production data, and establishment of mini-nurseries for fingerling rearing through community participation. The strategy of fingerling production in mini-nurseries is shown in Figure 5, from which it can be seen that there are three stages in the CBF strategy. First, induced breeding of exotic carp species and nursing of post larvae to fry stage are performed in the state owned AqDCs, secondly, community-based fry-to-fingerling rearing in mini-nurseries and in net cages installed in some irrigation reservoirs, and finally stocking fish fingerlings in small village reservoirs (mostly non-perennial) and minor, medium and major perennial reservoirs. Although, fry-to-fingerling nursing is performed in state-owned AqDCs, the maximum capacity of production is limited to 50 million fingerlings per year, which is not sufficient to stock most reservoirs of the country.

Due to these concerted efforts to increase inland fish production through development of CBF, there had been a dramatic increase in the total inland fish production from about 36,700 tonnes in 2000 to 57,410 tonnes in 2010 and then to 101,810 tonnes in 2020 (Figure 3).

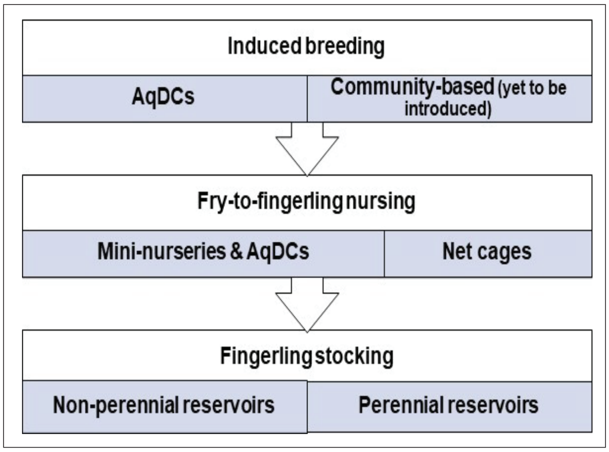

Figure 5: Institutional linkages for different stages of production of stocking material

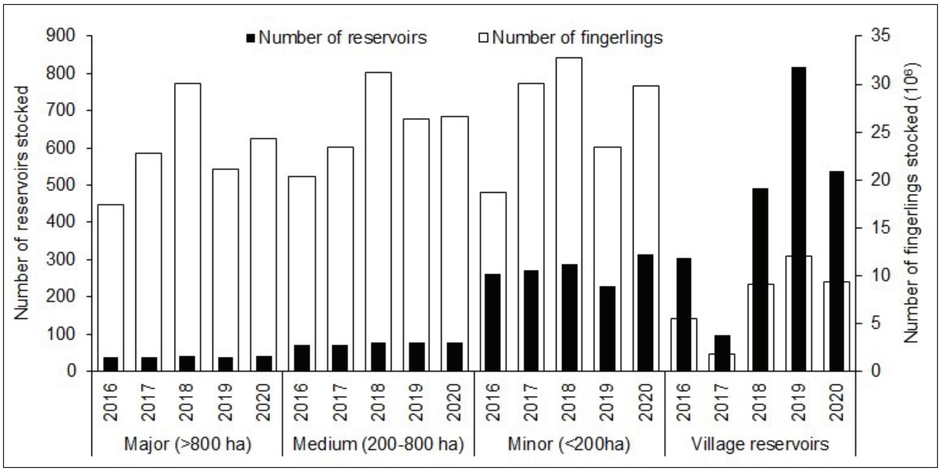

Figure 6: Number of reservoirs of different categories and number of fingerlings stocked during 2016–2020. White bars: number of fingerlings stocked; dark bars: number of reservoirs stocked. (source of data: NAQDA)

Fingerling production in mini-nurseries

CBF development in different kinds of reservoirs has become a regular inland fisheries development strategy in the country, and the greatest potential for CBF development exists in non-perennial reservoirs (Amarasinghe & Nguyen, 2010). Hence, availability of sufficient number of fingerlings for stocking non-perennial reservoirs at the correct time is a major constraint to CBF development in the country. When fingerlings are required for stocking one category of reservoir (e.g., minor perennial reservoirs), fingerling requirement for CBF development in non-perennial reservoirs is severely constrained due to their limited supply (Figure 6).

Under the ARDQIP, this issue had been addressed and there were initiatives to establish mini-nurseries through community-based organizations (CBOs) to fulfil the demand for fingerlings for CBF. Through this initiative, initial capital investment was borne by ARDQIP on the condition that CBOs must pay back the total amount in 60 instalments to NAQDA (Anon., 2006). At present, there are about 29 mini-nurseries in different parts of the country. They have been established by rural CBOs, mainly AqMCs. Almost all these mini nurseries are established as earthen pond systems, for which technical advice was provided to CBOs by NAQDA. Fish fry purchased from AqDCs of NAQDA are reared up to fingerling size in these mini-nurseries, which in turn are sold to CBF farmers. As the profit that the owners of mini-nurseries can earn is very significant, this strategy is bound to continue.

Freshwater prawn stocking

NAQDA's freshwater prawn hatchery, established in 2008 at Kahandamodara in southern Sri Lanka under the assistance of FAO, has a production capacity of 17 million post larvae per year. In addition, freshwater prawn breeding centre established in 2017 in Kallarawa, eastern province, has a capacity to produce 25 million post larvae per year. In the freshwater prawn hatchery in Pambala, north-western province, 13.5 million post larvae were produced in 2020. Although aquaculture technology of *M. rosenbergii* is well-developed elsewhere, such technology has not yet been effectively transferred to Sri Lanka. As a result, there is an over-supply of *M. rosenbergii* post larvae from the NAQDA's hatcheries. Freshwater prawn post larvae were therefore stocked in different kinds of reservoirs.

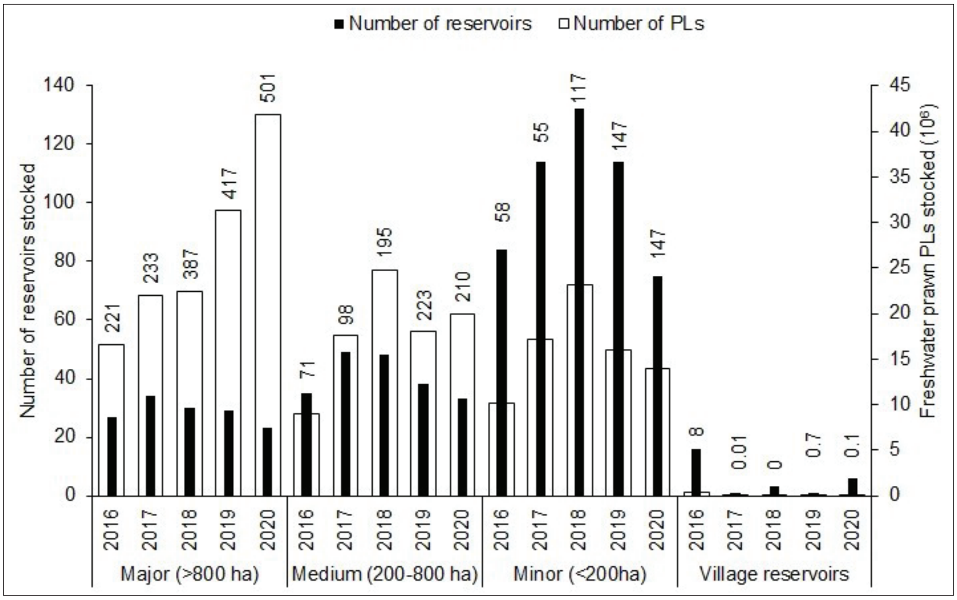

Figure 7: Number of reservoirs of different categories and number of freshwater prawn post larvae (PLs) stocked during 2016–2020. White bars: number of PLs stocked; dark bars: number of reservoirs stocked; numbers above columns indicate cumulative freshwater prawn yield per annum in tonnes (source of data: NAQDA)

Figure 8: Umbrella-shaped traps that are used in Thai reservoirs to catch freshwater prawn (Curtesy: T. Jutagate, Ubon Ratchathani University, Thailand)

The stocking data and reported yields of *M. rosenbergii* in different types of reservoirs in Sri Lanka during 2016-2020 (Figure 7) indicate that there is potential for incorporating freshwater prawn into CBF development in the country. Jutagate & Rattanachai (2010) reported that in Pak Mun reservoir (a run-of-river type reservoir of about 185 km^2 surface area) in Thailand, stocking of 2 million post larvae of *M. rosenbergii* resulted in an annual yield of 3 kg ha^{-1} and by stocking 40 million post larvae in 2003, a yield of 11.5 kg ha^{-1} yr^{-1} was achieved. Jutagate & Kwangkhang (2015) also reported that in Thailand, 30-day old post larvae of *M. rosenbergii* have been released into inland waters nationwide since 1980s for stock enhancement purposes. With the stocking density of about 2,500 prawn larvae/ha, harvests with an average individual weights ranging from 100 to 200 g after a year of release could be achieved. Common fishing gear used were gillnet, and traps that were designed exclusively for *M. rosenbergii*. However, the recapture rate was generally less than 5 %. Nevertheless, the economic return was high, which was about 3 times more than the average price of marketed freshwater fish (Jutagate & Kwangkhang, 2015).

In Sri Lankan reservoirs, fishers do not use special fishing methods to catch freshwater prawns but they are caught as by-catch in gillnets targeting finfish species. It would therefore be possible to introduce horizontal cylindrical traps (Deap *et al*., 2003) for catching *M. rosenbergii* in reservoirs where they have been stocked, which might increase freshwater prawn yields in reservoirs. Also, in Thai reservoirs, fishermen devised an umbrella shaped trap for catching *M. rosenbergii* in reservoirs (Figure 8) the efficiency of which was apparently high (T. Jutagate, pers. comm.). Although the freshwater prawn yield was much lower than finfish yields in CBF activities in Sri Lankan reservoirs, there were obvious financial benefits to the fishers because of high farm-gate price of freshwater prawn (LKR 570 – 705) compared farm-gate price of LKR 150-200 for freshwater finfish species.

Co-management

Co-management is essentially the sharing of responsibilities of decision-making and responsibility for the management of resources between the community (local fishers) and government centralised management (Pomeroy & Berkes, 1997). According to Brown *et al.* (2005), four pillars of successful co-management are as follows:

1) An enabling policy and legal framework
2) Participation and empowerment of communities (and other users).
3) Effective linkages and institutions.
4) Resources – a resource worth managing and the people and money to do it.

The CBF development strategies in Sri Lanka consist of these characteristics. As shown by Amarasinghe & Nguyen (2010), there are legal provisions to develop CBF in inland water bodies of the country under the Agrarian Development Act of 2000. Also, CBF development is a high priority area of fisheries development agenda of the country (Anon., 2007). CBF in village reservoirs are conducted by FOs (agrarian communities) rather than fishers. In medium and major reservoirs, CBF is practiced by fishers, who are also organised into rural fisheries organizations (RFOs). Community-based aquaculture (for fingerling rearing in mini-nurseries) is also within the organisational mandate of RFOs, whenever a farmer community decides to adopt it. The FOs are established under the Agrarian Development Act of 2000. The legal provisions in this Act facilitate empowering communities. The residents of a village involved in agriculture are essentially members of FOs. The small village reservoirs, whose command areas are less than 80 ha, come under the jurisdiction of Department of Agrarian Development and those which have irrigable area greater than 80 ha are under the control of Irrigation Department. Divisional Agriculture Committees (DvACs) are the major district-level institutions, which are responsible for facilitating agricultural development. There are monthly meetings of DvACs and the Divisional Secretary presides the meeting. Regional Officer (RO) of the Department of Agrarian Development, irrigation engineer, Samurdhi officer (government officer responsible for implementing state-sponsored poverty alleviation programmes), 'Grama Niladhari' (village-level administrative officer), aquaculture extension officer are the middle level government officers in this committee. Agrarian Research and Production Assistants (ARPAs), who work under the supervision of District Officer (DO), are the major contact persons of FOs. The officer bearers of FOs and ARPAs are also participants of monthly meetings of DvAC (Amarasinghe, 2010; Amarasinghe & Nguyen, 2010). These institutional linkages provide a positive environment for introduction of co-management strategies for CBF.

Extensive availability of village reservoirs, which are not used for fisheries development, willingness of agricultural communities to take up CBF, and well-established CBOs for operating mini-nurseries for fingerling rearing are the resources prevailing for CBF development in the country. In some reservoirs, CBF committees have invested money to establish computer-assisted data bases for stocking, harvesting, marketing and welfare fund mobilisation, due to promising financial benefits from CBF. Members of CBF committees become partners of community-based enterprises and they invest in the community owned venture. Also, members share the financial benefits of CBF activities on an agreed basis, which ensures sustainability of the strategy. These features in CBF strategies in Sri Lankan reservoirs indicate potential for establishing co-management effectively. In fact, such co-management strategies exist in several reservoirs of the country (Chandrasoma *et al*., 2015).

Pushpalatha *et al.* (2020) used Ostrom's modified design principles (Ostrom, 1990; Cox *et al*., 2010) as benchmarks to evaluate the strategies developed by fisher communities in five irrigation reservoirs for institutional robustness and resource management. Results indicated that increased levels of compliance with design principles through improving leadership qualities and empowering fishers for management decision-making would ensure sustainability of CBF due to high economic gains of CBF communities in reservoirs (Pushpalatha *et al*., 2020). Also, Pushpalatha *et al.* (2020) have shown that in five irrigation reservoirs of Sri Lanka, there was a multi-layer institutional structure, which links fisher communities between reservoirs (horizontal integration) and with fisheries organisations at the district level, provincial level and at the national level (vertical integration). Such kind of a multi-layer institutional structure as depicted in Figure 9, is recommended to be established for effective co-management of CBF in Sri Lankan reservoirs.

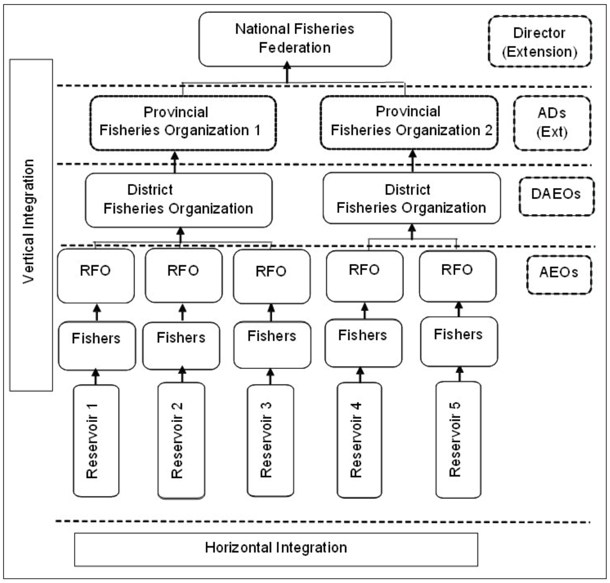

Figure 9: Multi-layer institutional structure, which links rural fisheries organisations (RFOs) between reservoirs (horizontal integration) and with fisheries organisations at the district level, provincial level and at the national level (vertical integration). The multi-layer institutional structure in NAQDA includes aquaculture extension officers (AEOs) in the RFO level, district aquaculture extension officers (DAEOs) in district level, assistant directors of extension [ADs (Extension)] in provincial level, and Director (Extension) at the national level.

Constraints to CBF development

Most of small village reservoirs dry up almost entirely during the dry season in August-October. Utilization of them for CBF development should therefore be planned essentially depending on the inter-monsoonal rains, generally during December-January period. It is therefore necessary to make fingerlings available for stocking reservoirs at the correct time. Consequently, the strategy should involve correct timing of fry-to-fingerling rearing, and induced breeding of Chinese and Indian major carps in the hatcheries (Wijenayake *et al*., 2021). The highest number of non-perennial reservoirs stocked in a year over the period of 2016 – 2020 was 819 (Figure 6), which was approximately 7% of total number of small village reservoirs in the country. As such, opportunities exist for further development of CBF in small village reservoirs of Sri Lanka. However, the full potential of utilization of these water bodies for CBF development is not achieved mainly due to the inadequacy of stocking material at the correct time under the present strategy of fish fingerling production for stocking reservoirs. It is therefore imperative that effective intervention be in place for the establishment of backyard hatcheries using simple technologies to the self-help community groups in the rural areas. Mohapatra *et al.* (2015) reported a case study of introduction of a portable fibreglass reinforced plastic (FRP) hatcheries along with technical support to a remote and inaccessible village in Odisha, India targeting women self-help groups. In this case, a poor group of women with no ownership of such assets was able to produce fish seed in a remote area. Through the government extension mechanism, an enabling environment was provided which contributed to the group having sufficient confidence for successful operation of the hatchery. It would be possible that through 'technology transfer research', small-scale carp hatcheries can be established in rural areas of Sri Lanka, as practiced in rural villages in Odisha, India.

Pushpalatha (2020) reported that in some irrigation reservoirs, semi-circular barrier nets were installed in front of the sluice gates to prevent escape of stocked fish fingerlings when water is released for irrigation for downstream paddy cultivation. According to Pushpalatha (2020), substantial increase in the CBF yield was evident in several perennial reservoirs where such barrier nets were installed. However, economic feasibility of this strategy is yet to be evaluated. In Chinese reservoirs, where CBF are practiced, fish escape prevention devices such as barrier nets and electrical barriers have been reported to be established (Xu, 1983; 1985).

Wijenayake *et al.* (2021) have shown that due to anomalies in monsoonal rainfall patterns under climate change scenario, water availability in non-perennial reservoirs in the dry zone of Sri Lanka for CBF development is rather unpredictable. As such, there is an uncertainty that rural groups engaged in fry-to-fingerling production face the difficulty of selling fish fingerlings to CBF farming groups when the non-perennial reservoirs are not filled due to insufficient monsoonal rains. An alternative strategy to link fingerling-producing community groups with the agricultural farming communities in minor perennial reservoirs should be in place to prevent such potential financial losses of fingerling producers (Wijenayake *et al*., 2021).

Opportunities for further development

The gradual success of CBF based on the application of science and improved community organisation sparked off an ancillary sector of fry to fingerling rearing bringing about employment opportunities and economic gains to rural households. When the feasible solutions are adopted to address constraints such as fingerling scarcity and those associated with climate change anomalies, there are promising future prospects of achieving full-scale CBF production. It is a reality that consumer preference in Sri Lanka for freshwater fish is generally low compared to marine fish. This is particularly so for exotic carp species, which significantly contribute to CBF yields in reservoirs. As such, at full-scale CBF development in Sri Lanka reservoirs, there is a potential to create a glut in the local market. Through the initiative by NAQDA, a fish canning company started producing three products of canned fish from catla flesh. As a new demand has arisen due to these fishery products, farmgate price of Indian carps in many reservoirs has elevated. Also, there are more popular postharvest fishery products available in the market (James, 1998). These include, surimi, fish mince-based products, fish cutlets, fish burgers, fish fingers, fish protein extract, etc. For this purpose, research on postharvest product development using freshwater fish including market research is needed to be carried out.

The primary uses of reservoir resources in Sri Lanka are irrigation of agricultural lands and hydroelectricity generation. Inland fisheries development is essentially a secondary use of reservoirs. As a result, there are many constraints to inland fisheries development due to the multiple uses. Hence, potential conflicts of interest in the inland fishing industry due to different multiple uses should be minimized. A synergy between water management related to downstream activities and fisheries enhancement should therefore be ensured for sustainability of CBF. A new act named as 'Aquaculture and Culture-based Fisheries Act' is proposed. There are several provisions under this proposed Act for multi-sectoral coordination, under which there shall be a culture-based fisheries management coordinating committee. This committee is proposed to consist of officials of various government institutions having responsibilities for the management of reservoirs and their catchments.

De Silva (2003) mentioned that CBF is an environmentally friendly form of aquaculture. It would therefore be possible to acquire global recognition for

CBF through implementation of the FAO code of conduct for responsible fisheries through an ecosystem approach to fisheries and aquaculture (Staples & Funge-Smith, 2009). This will also help achieving balance between conservation and fishery resources enhancement.

CONCLUSIONS

Sri Lanka is endowed with a multitude of freshwater reservoirs most of which are very small (< 50 ha) village irrigation reservoirs. These small village reservoirs are not capable of supporting any commercial scale fisheries through natural recruitment, although biologically very productive. As such, there is a high potential for the development of culture-based fisheries (CBF) in Sri Lankan reservoirs. The efforts made in 1980s to develop CBF in these water bodies were disrupted due to lack of proper scientific approaches for selection of suitable reservoirs. Under a comprehensive research project, a best practice approach was developed to select suitable reservoirs for CBF development using multi-criteria decision-making procedure. Social mobilisation and development of entrepreneurship plan among rural communities ensured sustainability of CBF in many reservoirs. Also, there were positive initiatives of fisheries authorities such as establishing mini nurseries for rearing of fingerlings of Chinese and Indian major carps, pilot scale CBF development in minor perennial reservoirs and establishing effective data recording systems. As detailed out by Chandrasoma *et al.* (2015) CBF has been successfully extended to medium and major perennial reservoirs that have resulted in significant increases in fish yield, and consequent gains in the socio-economic status of fishers and related communities. Increased fish yields through CBF activities also have expanded the associated value and market chains benefiting rural communities through added employment opportunities. Recent attempts at stocking *Macrobrachium rosenbergii* post larvae in inland reservoirs as part of CBF contributed to significant elevation of rural income. In many rural communities, there had been remarkable trends that part of the profit earned by CBF had been mobilised for various welfare activities in the village. Opportunities exist for establishment of effective co-management strategies for CBF in irrigation reservoirs of Sri Lanka. Being a rural development strategy, CBF development in Sri Lankan reservoirs should essentially be a transdisciplinary approach. Such an approach should take into consideration of the biological productivity favouring growth of stocked fish, institutional linkages for supporting different stages of the strategy, social attitudes for adopting CBF within the realm of participatory development, and market forces driving the entire process.

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REVIEW

Molecular logic-based computation and fluorescent sensors: The story so far

CY Yao and AP de Silva*
School of Chemistry and Chemical Engineering, Queen's University, Belfast BT9 5AG, Northern Ireland.

Summary: The path to molecular logic-based computation began in Colombo, Sri Lanka and proceeded to Belfast, Northern Ireland. From there it spread to many cities around the world when over 1260 laboratories contributed examples and ideas to advance the field (Figure 1A-C). The formative experiments and some future directions are mapped out in this perspective. Principles discovered here should have a bearing on the computing performed continuously by molecule-based living things to survive and prosper. The more mature field of fluorescent sensors served as the springboard for molecular logic, and still serves a continuing need in society for monitoring atomic and molecular species in various situations for improving health, environment and economy.

Keywords: Blood electrolyte analyzer, fluorescent PET sensing/switching, molecular logic-based computation, photoinduced electron transfer.

INTRODUCTION

A powerful approach for relaying atomic and molecular information to us humans relies on 'off-on' fluorescence signalling of molecular sensors (Bryan *et al*., 1989; de Silva *et al*., 1997; Lackowicz, 2006; Demchenko, 2009; de Silva, 2011a; Valeur & Berberan-Santos, 2012). These designed molecules can infiltrate living cells which are also molecule-based. Then the private world of atoms in living cells (Tsien, 1992; de Silva *et al*., 1999) and tissues (de Silva *et al*., 1999) becomes no longer private. Such gathering of information illuminates physiology and enables diagnostics for medical purposes. Extension of this idea leads to fluorescent signalling systems, which respond to different sets of atomic/molecular species. Input-output patterns of this type fit the logic gates described by George Boole's students in the 19th century (Boole, 1958; Malvino & Brown, 1993; Gregg, 1998) and which are exploited in modern semiconductor-based information technology. The first experimental approach to molecular computation arose in this way (de Silva *et al*., 1993) and serves to connect chemistry to biological sciences and to computer science.

COMMUNICATING BETWEEN WORLDS

Light signalling from the molecular world to ours requires careful design of the signaller according to engineering principles. The design arose as follows. One of the authors was fortunate to be exposed to the principle of photoinduced electron transfer (PET) (Weller, 1968) during his PhD student days under the supervision of Jim Grimshaw at Queen's University Belfast (Grimshaw & de Silva, 1980; 1983). Ajita Abeysekera had pioneered the Colombo-Belfast nexus during his PhD work with Ron Grigg (Abeysekera *et al*., 1976a; 1976b; 1977a; 1977b; 1979; 1980; 1985). After returning to Colombo, one of the authors found that PET could be arranged within a 'fluorophore-spacer-receptor' system so that the fluorescence was switched 'off'. This turned out to have substantial precedent, but the recovery of fluorescence to an 'on' condition was rare (Wang & Morawetz, 1976; Selinger, 1977; Shizuka *et al*., 1979; Konopelski *et al*., 1985). Although not previously recognized as such, 'fluorophore-spacer-receptor' systems were hiding in plain sight within anti-malaria drugs (Bissell *et al*., 1992a). Our first reported case **1** was modelled on these (de Silva & Rupasinghe, 1985). Here, an amine group served as a receptor for H^+. Thus, we had a new class of fluorescent

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* Corresponding author (a.desilva@qub.ac.uk; https://orcid.org/0000-0003-4914-1333)

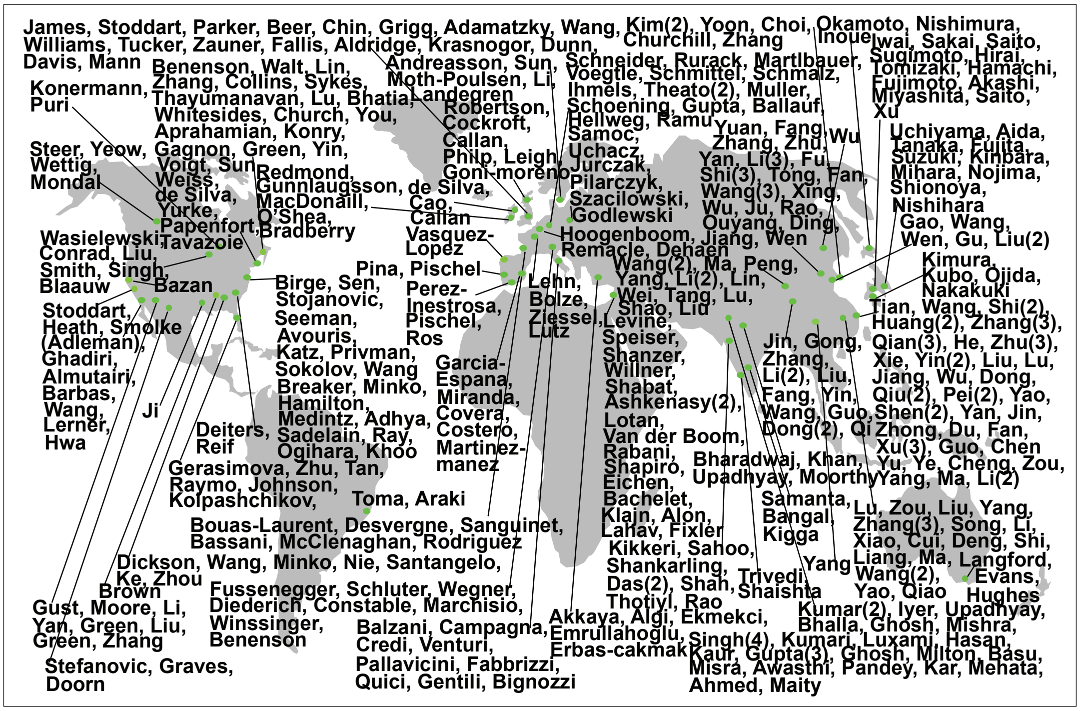


Figure 1A: Approximate world maps of some sources of molecular logic devices and cases which are understandable as such. In all cases, only the names of corresponding authors from the literature are given. The simplest cases of single-input, single-output binary logic devices are not included.

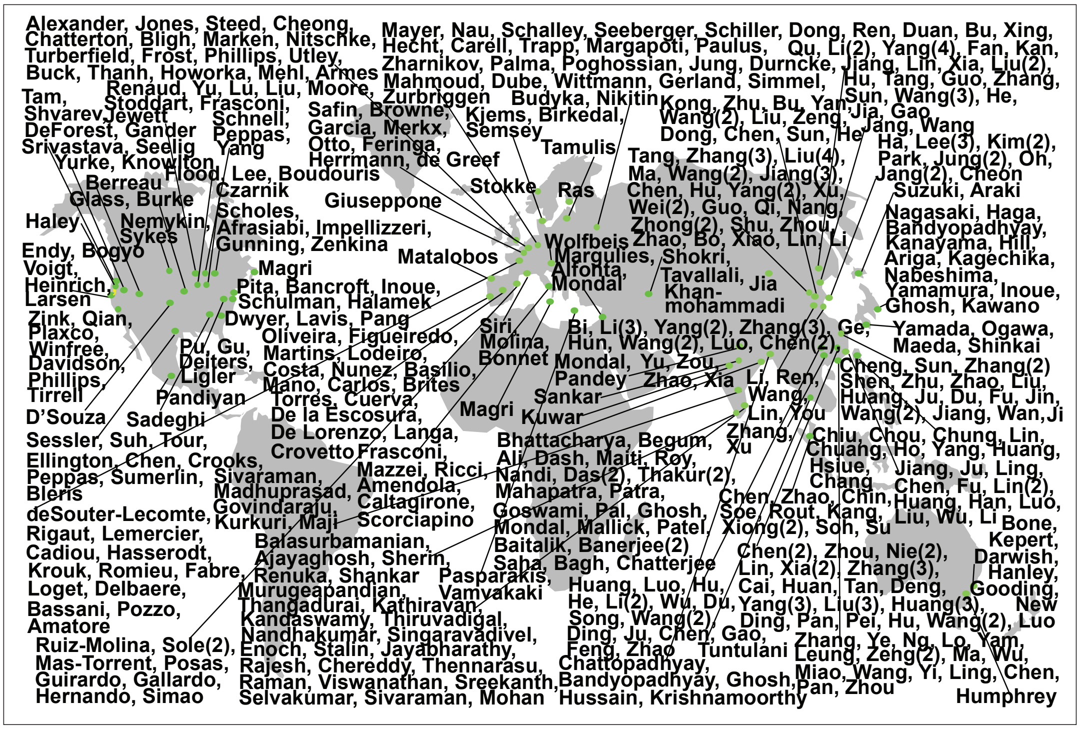


Figure 1B: Approximate world maps of more sources of molecular logic devices and cases which are understandable as such.

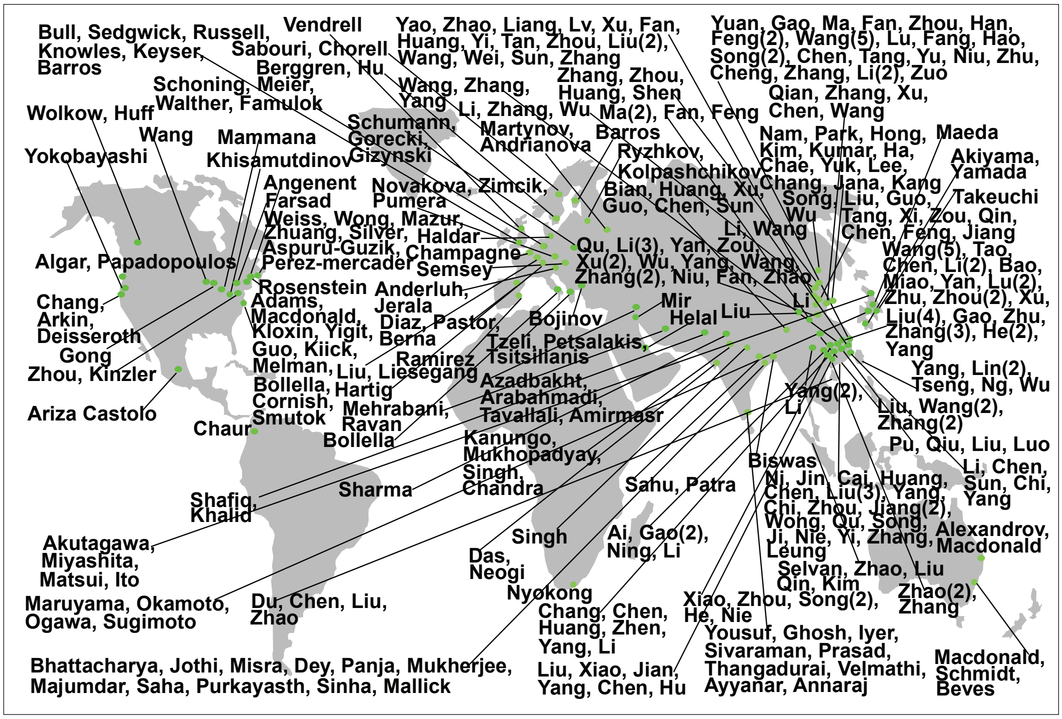


Figure 1C: Approximate world maps of further sources of molecular logic devices and cases which are understandable as such.

pH indicators on our hands. Perhaps pH indicators themselves did not need additions, since innovative uses continue to emerge (Wang & Anslyn, 2011) for these venerable compounds (Bishop, 1972). However, the 'off-on' switching of fluorescence, the modular engineering design and the resulting predictability of several electronic spectroscopic parameters (de Silva *et al*., 1989) promised that fluorescent PET sensors had launched with protons and that more would follow. Indeed, it was quickly demonstrated that Na^+ could be sensed in a similar way simply by replacing the amine with an azacrown ether (Konopelski *et al*., 1985; de Silva & de Silva, 1986). Other exploitations of modularity were also made for sensing applications (de Silva *et al*., 2007). As far as we can discern, over 950 laboratories around the world have contributed to fluorescent PET sensors thus far (Figure 2).

The fluorescent 'off-on' signalling mechanism deserves elaboration. When the amine receptor of **1** becomes protonated, its oxidation potential rises. So the PET process becomes energetically expensive. The previously allowed electron transfer becomes disallowed, so that the excited state de-excites via fluorescence emission (de Silva *et al*., 1997; Daly *et al*. 2015a; Magri, 2015). Such an increase of a redox potential in the presence of a cationic input species, e.g. Na^+, was later found to be rather general (Beer *et al*., 1989; Kenmoku *et al*., 2004).

The engineering design suggested that more modules could be added to 'fluorophore-spacer-receptor' systems to endow them with new properties. However, this had to wait until one of the authors had returned to Belfast. Nevertheless, University of Colombo put its stamp on this process in two ways. Satish Namasivayam gave instruction on logic gate hardware. Gihan Wickremanayake and Kevin Seneviratne passed on computer programming knowledge. These valuable inputs prepared us to link the worlds of computing and of chemistry/biology in a new way. Computational chemistry and computational biology employ conventional semiconductor computers to solve problems in these fields (Kohn, 1999; Pople, 1999). However, now we have chemical computation and biological computation, where chemical and biomolecular systems perform the computation itself. This should not be too surprising since living things have always processed information from their environment in order to survive and prosper. Here lies the original information technology.

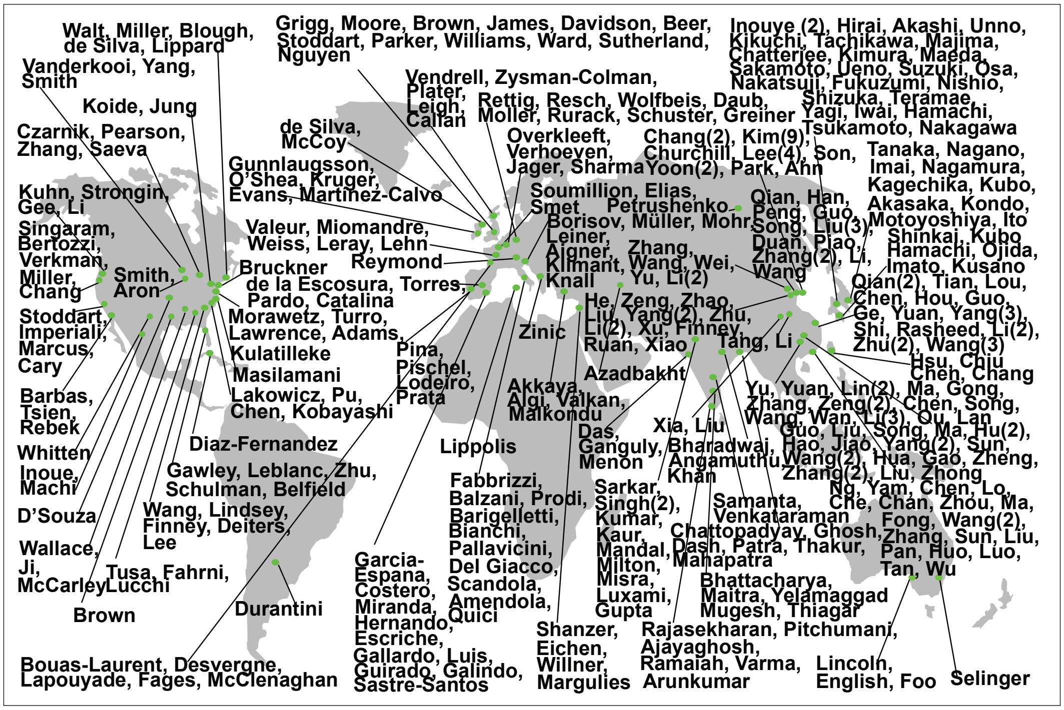


Figure 2A: Approximate world maps of some sources of fluorescent PET sensors and switches and cases which are understandable as such.

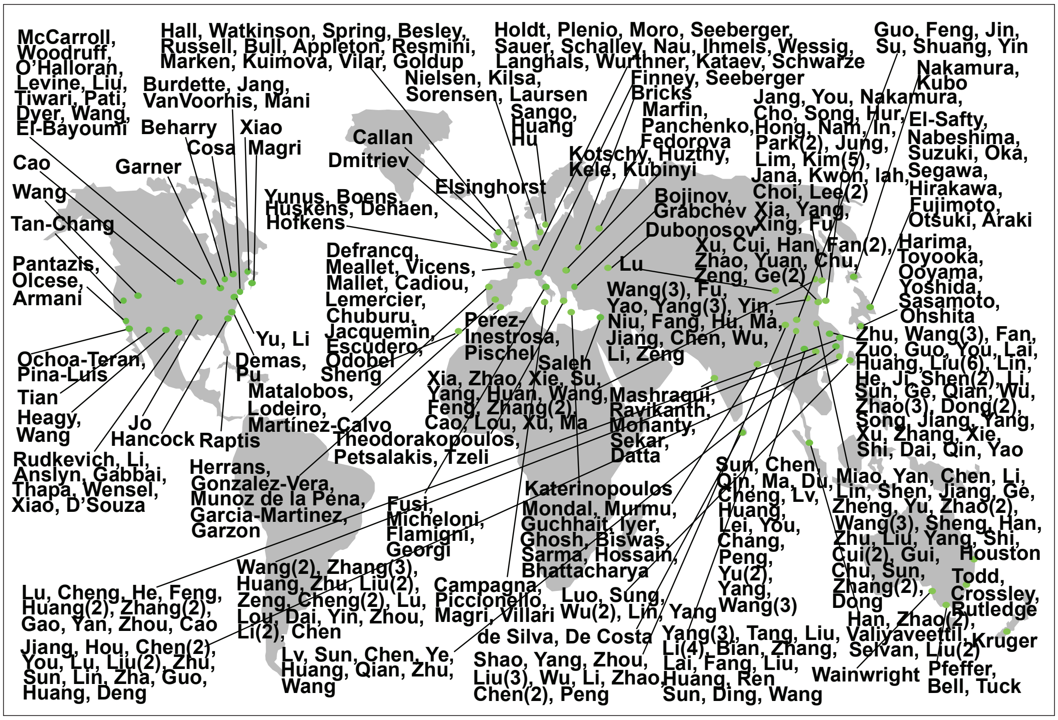


Figure 2B: Approximate world maps of more sources of fluorescent PET sensors and switches and cases which are understandable as such.

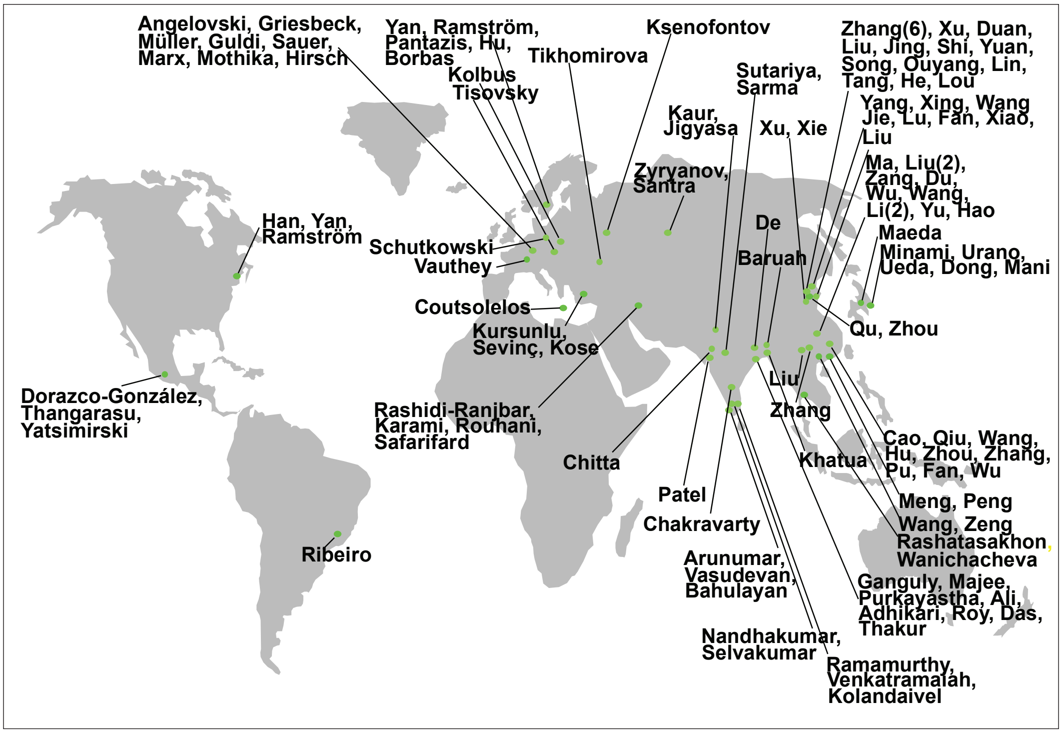
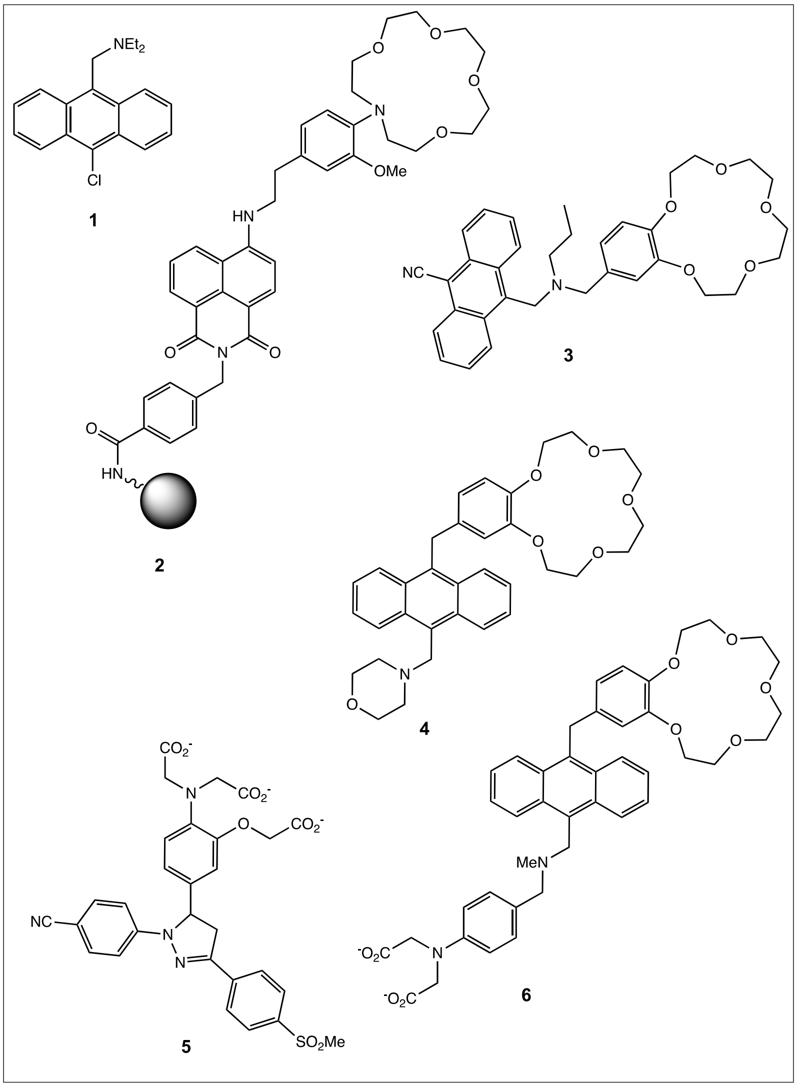


Figure 2C: Approximate world maps of further sources of fluorescent PET sensors and switches and cases which are understandable as such.

Sensing for lifesaving

Molecular computing (Carter *et al*., 1988; Brown *et al*., 2002; de Silva *et al*., 2006; 2007; Magri *et al*., 2007; 2021; de Silva & Uchiyama, 2007; Balzani *et al*., 2008; Feringa & Browne, 2011; de Silva, 2011a; 2011b; 2013; Szacilowski, 2012; Katz, 2012a; 2012b; 2019; Daly *et al*., 2015b; Andréasson & Pischel, 2018; Erbas-Cakmak *et al*., 2018; Yao *et al*., 2020) at its simplest level concerns single input-single output devices. In Boolean language, this amounts to YES or NOT logic (Malvino & Brown, 1993; Gregg, 1998). The former has the output following the input whereas the latter has the output opposing the input instead. Compounds like **1** satisfy YES logic because 'high' levels of H^+ cause 'high' levels of fluorescence intensity. Thus, YES logic gates serve as sensors. We were challenged by the giant multinational Roche to develop a similar compound for tracking Na^+ in whole blood (He *et al*., 2003; Tusa & He, 2005). An important practical obstacle was removed by passing the blood through a micron filter so that the serum could be interrogated with a blue light-emitting diode. Polymer-bound compound **2** satisfied the challenge since its aminonaphthalimide fluorophore (Qian *et al*., 1989; Alexiou *et al*., 1990; de Silva *et al*., 1995; Zheng *et al*., 2012) absorbed blue light effectively and emitted green-yellow light in the presence of 'high' Na^+. Its N-aryl-azacrown ether receptor captured Na^+ while rejecting H^+ at neutral pH. Importantly, this receptor also carried a OMe sidechain which improved the size-match with Na^+ while rejecting the larger cation, K^+, at its usual concentration in blood (Schultz *et al*., 1985). Also, the binding constant for Na^+ in water (ca. 10 M^{-1}) for this receptor is ideal for sensing Na^+ concentrations around the normal value in blood (0.1 M). The OPTI blood electrolyte analyzer, built around fluorescent PET sensors like **2**, has been deployed around the world in hospitals, ambulances, general practice surgeries, as well as veterinary situations, for over 20 years now (*www.optimedical.com*; *www.idexx.com*). A particular instance of lifesaving is where ambulance crews can inform the hospital of a patient's blood Na^+ level so that a blood bag can be suitably prepared in order to avoid salt shock after transfusion.

Simpler fluorescent YES logic gates based on PET, like **1**, also serve to monitor the after-effects of radiotherapy (Paglin *et al*., 2001) and to track acidic lysosome compartments inside living cells (*www.thermofisher.com*). The results of such experiments with fluorescence microscopes also feed back into healthcare applications of the near future.



From one input to two

One of the strengths of modular systems is the ease with which extra modules can be added (Bissell *et al.*, 1992b; de Silva *et al.*, 2008). For instance, a 'fluorophore-spacer$_1$-receptor$_1$-spacer$_2$-receptor$_2$' system carrying orthogonal receptors could enable a PET process to be launched from either receptor to quench emission from the fluorophore. Then, both receptors need to be occupied by their respective target species before PET processes are fully disabled. Fluorescence switching 'on' becomes possible only at that stage. According to Boolean ideas, this would be AND logic behaviour, since output becomes 'high' only when both inputs are separately 'high' (Gregg, 1998; de Silva, 2013). Compound **3** extends the structure of YES logic gate **1** with an extra methylene spacer and a benzocrown ether. The latter is reminiscent of compound **2**, so that the fusion of two YES gates driven by H$^+$ and Na$^+$ respectively produces a H$^+$,Na$^+$-driven AND gate (de Silva *et al.*, 1993). A 'receptor$_1$-spacer$_1$-fluorophore-spacer$_2$-receptor$_2$' system is another permutation of these modules where the distances involved in the PET processes are minimized (de Silva *et al.*, 1997). With these faster PET rates, the ratios of fluorescence intensity output between the 'on' and 'off' states become larger. This makes for more distinct AND gate performance in compound **4**.

AND is only one of 16 logic types which deal with two inputs and one output (Gregg, 1998; de Silva, 2013). For instance, OR logic produces a 'high' output signal whether one or other input is 'high'. A molecular illustration of this is presented in 'fluorophore-spacer-receptor' system **5**, where the amino acid receptor binds Ca^{2+} strongly or Mg^{2+} moderately to suppress PET and switch 'on' fluorescence in either case if sufficiently high concentrations of either cation are applied. Examples of each of the 16 types are now available from various laboratories to demonstrate how all possible combinations of two species of 'high' or 'low' concentrations can be sensed (de Silva, 2013; Yao *et al*., 2020).

From two inputs to three…

Continuing the modular theme (Bissell *et al*., 1992b; de Silva *et al*., 2008), the system 'fluorophore-spacer$_1$-receptor$_1$-spacer$_2$-receptor$_2$' can be extended to a 'receptor$_3$-spacer$_3$-fluorophore-spacer$_1$-receptor$_1$-spacer$_2$-receptor$_2$' version while ensuring orthogonality among the three receptors. As before, these modules can be chosen so that a PET process originates from each receptor to quench emission from the fluorophore. Then, all three receptors need to be blocked by their respective target species before PET processes cease and intense fluorescence emerges. This is three-input AND logic action (Gregg, 1998; de Silva, 2013). Although this extension might seem trivial in a mathematical sense at first sight, it is worth remembering that a change from two to three is not at all trivial in several other contexts. Even in decimal mathematics, a two-number password can be guessed in a maximum of 100 attempts whereas a three-number password would require a much larger number of tries (1000). In the natural sciences, a change from two dimensions to three gives the occupant freedom to fly and jump instead of crawling or walking along a surface. In medicine, detecting the presence of three chemical targets simultaneously instead of two can sharpen the detection of a disease, e.g. cholesterol, low density lipoprotein and C-reactive protein indicate heart problems.

Compound **6** is a forerunner of 'lab-on-a-molecule' systems (Magri *et al*., 2006) where simultaneously 'high' levels of Na^+, H^+ and Zn^{2+} are signalled by a switching 'on' of fluorescence. 'High' levels are defined as being significantly larger than the reciprocal of the corresponding binding constant of the input ion with **6**. Orthogonality among the benzo-15-crown-5, aliphatic amine and N-aryl amino acid receptors are enhanced by limiting the pH range to around neutrality. As suitably selective receptors continue to be discovered, they can be co-opted into these 'lab-on-a-molecule' systems to deliver a single 'yes' or 'no' answer for diagnosis of certain illnesses. The alternative of performing three separate tests and then considering the three results would take longer besides requiring an intervention by a professional. 'Lab-on-a-molecule' systems (Magri *et al*., 2006; 2014; Chen *et al*., 2015; Scerri *et al*., 2019) should be suitable for rapid diagnosis of disease, especially in a post-pandemic world where the public remembers overstretched health systems only too well.

Putting memory into molecular logic-based computation

Everyday computers have stored programs or applications which perform a specific set of functions, such as those needed to compose a document, to produce a spreadsheet or to order a meal from a restaurant. A memory is an essential requirement in such situations. On the other hand, many information-processing tasks, such as those discussed in previous sections, require no memory. Computer scientists and electronic engineers distinguish between combinational and sequential logic (Malvino & Brown, 1993). The former has no memory whereas the latter does. Importantly, memories can be built from the same gates which are employed in combinational logic operations, if feedback loops are provided. For instance, the RS flip-flop is a common memory unit which is constructed from two NOR gates which are cross-wired for feedback purposes (Figure 3) (Malvino & Brown, 1993). A given output state, which is set-up by a specific input signal, can be maintained until such time that it is deliberately switched over to the other state by provision of another specific input signal.

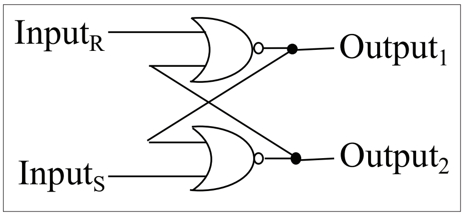
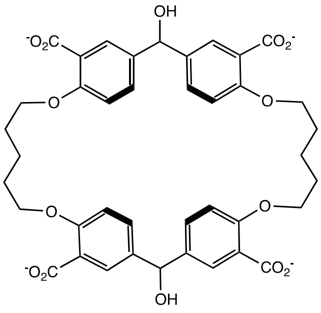


Figure 3: Physical electronic representation of the common memory unit, the RS flip–flop. The system is set by making Input$_S$ 'high' and Input$_R$ 'low', Output$_1$ becomes 'high' and Output$_2$ becomes 'low'. This output situation is maintained until the system is reset by making Input$_S$ 'low' and Input$_R$ 'high'. Then Output$_1$ becomes 'low' and Output$_2$ becomes 'high'. This output situation is maintained until the system is set again.

In the molecular sphere, such memories are available in the form of photochromic compounds (Irie, 2000). These can be set in a coloured state by uv irradiation, and returned to the colourless state by visible irradiation (Pischel & Andréasson, 2010). We chose a different route to molecular memories by using an alcohol state which is set by reduction, and returned to a ketone state by oxidation (Daly *et al*., 2019). By outfitting this system with a macrocycle, it was possible to arrange a large cavity in the alcohol state (**7**) whereas the cavity was constricted in the ketone state (**8**) by exploiting the pi-delocalization capability which is only present in the ketone. So we have the ability to capture a guest (**9**) in the alcohol state **7** and to release the guest from the ketone state **8**. Molecular-scale delivery can thus be accommodated in logic designs.

Additional logic functions can be integrated with the RS flip-flop because the guest occupancy within host **7** results in the loss of its fluorescence ability. This is due to PET occurring from the electron-rich phenylene walls of host **7** to the electron-poor aromatic unit within guest **9**. From a general viewpoint, here we have a fluorophore within the receptor which interacts with the bound guest in a pseudo-intramolecular fashion. An everyday analogy would be a taxi which indicates occupancy with a light signal on its roof.



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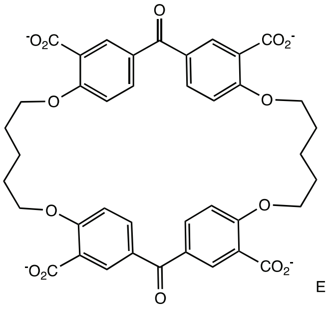

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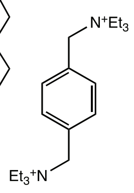

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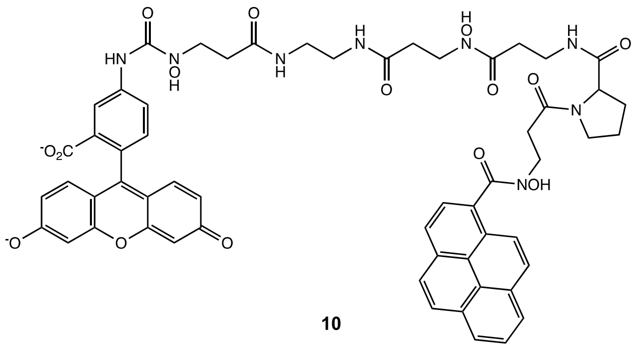
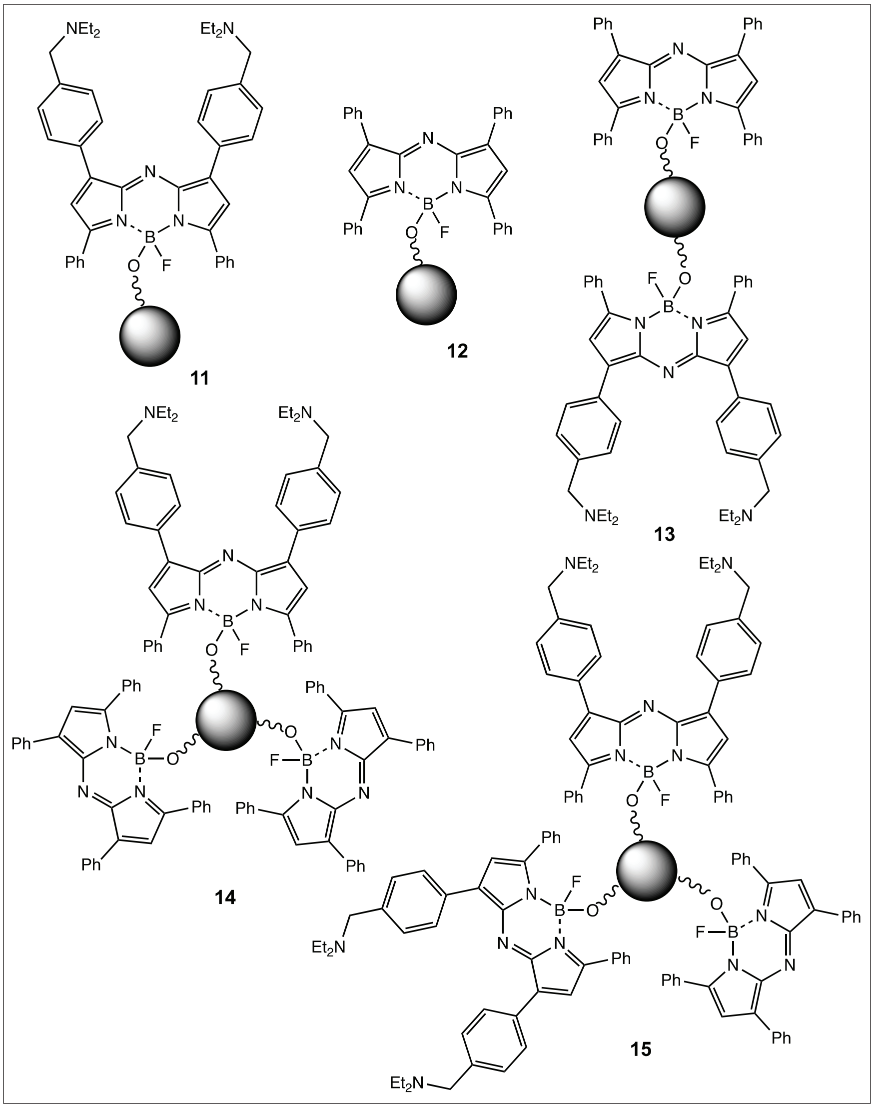


10

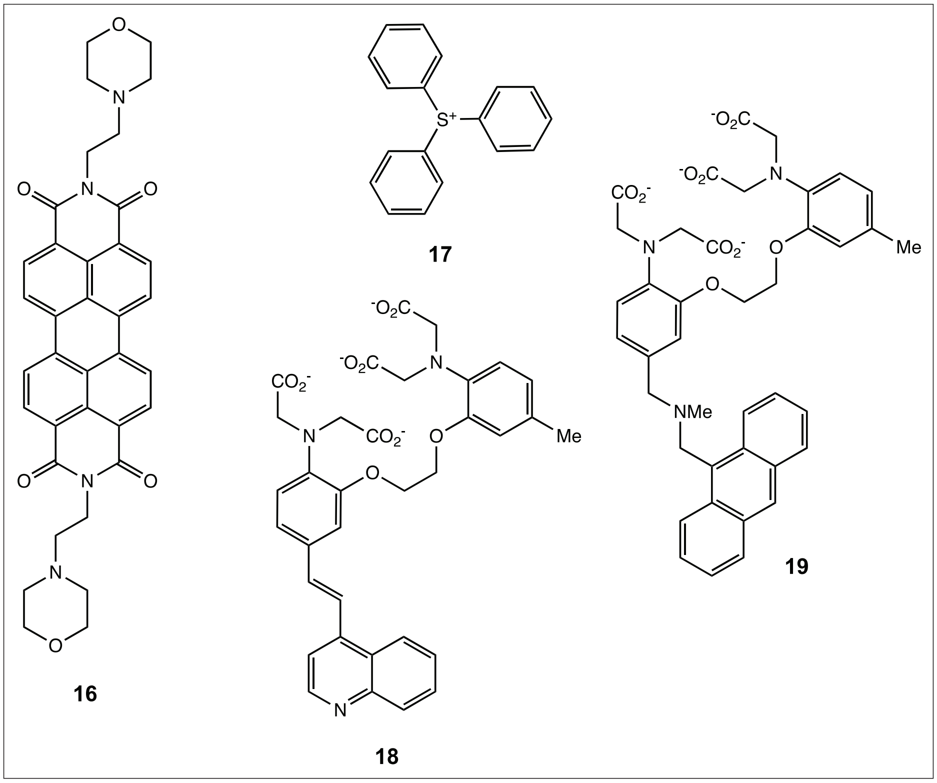
An important avenue of modern research in molecular logic - security (Andréasson & Pischel, 2018) was energized with compound **10** (Margulies *et al*., 2007). Generally, memory is essential for security applications since it needs to be remembered against whom the security is required and under what circumstances. A molecular keypad lock is a device where inputs need to be applied in the correct temporal sequence before a 'high' output signal is produced which can actuate a lock-opening. From an electronics viewpoint, such devices are essentially AND gates where one input is prioritized for being applied earlier than another. When complexed with Fe^{3+}, **10** is non-fluorescent owing to the open-shell nature of the metal ion creating PET and electronic energy transfer pathways for de-excitation. A 'high' green fluorescence signal emerges only if an acidic EDTA (ethylenediaminetetraacetic acid) $input_1$ is added first to extract the Fe^{3+} away from **10**, followed by the supply of an acetate $input_2$ to create the well-delocalized fluorescein fluorophore under the basic conditions and concluded by provision of near-ultraviolet light (which is counted as input$_3$) to excite this fluorophore. Other permutations of the input string fail to produce the 'high' fluorescence output under the strictly defined conditions. **10**, or versions thereof, has been exploited previously to build combinational logic systems (Margulies *et al*., 2004) and subsequently to develop hack-proof communication protocols (Sarkar *et al*., 2016; Lustgarten *et al*., 2019) as well as combinatorial sensors (Rout *et al*., 2014).

Useful molecular logic-based computations

An early challenge to the field of molecular logic-based computation was to provide possible solutions to currently unsolved problems. The fact that molecules are small and biocompatible cannot be matched by semiconductor devices, even though current transistor sizes are well in the molecular size range. This is because operational transistors always require wires or antennas to interface with the human world, besides semiconductors being different in nature to living systems. Such peripherals always add to the size of the working device system, as in radiofrequency identification for instance (Shepard, 2005). As pointed out above on several occasions, molecules can interface with the outside world by using light. The recent growth in intracellular computation by designed molecules (Bronson *et al*., 2008; Win & Smolke, 2008; Murale *et al*., 2013; Finkler *et al*., 2016; Grimm *et al*., 2016; Green *et al*., 2017; Matsuura *et al*., 2018; Eordogh *et al*., 2020; Zhang *et al*., 2021) has provided a powerful response that is bound to grow into the future. However, the first useful computation was demonstrated with inanimate objects.



Polymer beads of micrometric size are employed in so many applications that they are even considered a pollution hazard nowadays (Chen, 2020). One of these applications concerns their use as vehicles for compound libraries built-up by combinatorial chemistry (Wilson & Czarnik, 1997). Each bead requires a tag for its identification during tests for the efficacy of its compound in drug discovery for example. Fluorescent dyes can serve in this capacity but the broadness of molecular excitation/emission bands and the limited bandwidth in the visible spectrum combine to constrain the number of distinguishable tags to around a hundred (Smith Kline Beecham Corporation, 2001). Enhancing fluorescent dyes with logic capabilities significantly increases the number of distinct tags since various input species can be employed to trigger the fluorescence signal in various patterns satisfying different logic functions of bead-bound cases like YES (**11**) and PASS 1 (**12**) (de Silva *et al*., 2006; Brown *et al*., 2008; McKinney *et al*., 2017; Refalo *et al*., 2018; 2019; Yao *et al*., 2019). Attaching two logic tags to a bead multiplies the logic diversity even further. For instance, a YES gate and a PASS 1 gate can be attached to separate samples of beads in different ratios in order to produce distinguishable logic types, e.g. YES + PASS 1 (**13**), YES + 2PASS 1 (**14**), 2YES + PASS 1 (**15**), etc. Each of these can be identified within a population by fluorescence microscopy (Yao *et al*., 2019).



Computations that we do unconsciously

Although we might like to believe that we are continuously applying high intelligence, everyday living requires many decisions to be taken in the background. For instance, our eyes help to evaluate the threat potential of approaching objects (Bruce *et al*., 2003; Lazareva *et al*., 2012; Hock & Nichols, 2013). Within milliseconds, an image of the object is recorded and the image is processed into a set of lines which correspond to the detected edges. A significant reduction of the dataset is achieved in this way, so that it can be easily sent for comparison with a series of edges stored in the brain under the class of dangerous objects. If a match is found, our legs are actuated to flee the scene. Such suitable evasive action can ensure survival. All these computations are carried out by molecule-based systems within ourselves, and have been emulated by information technologists in recent years (Shapiro & Stockman, 2001).

Remarkably, such security operations can be conducted by carefully chosen mixtures of molecules **16** and **17** (Ling *et al*., 2015a). Compound **16** is a 'fluorophore-spacer-receptor' system whose fluorescence output responds to H^+ in a YES logical manner (Daffy *et al*., 1998). Compound **17** supplies the required H^+ when it is photoexcited (Dektar & Hacker, 1990). This allows a fluorescence to be switched 'on' anywhere which is illuminated by 254 nm writing light. However, the situation alters as the writing times are extended, since an electron-rich photoproduct accumulates. This quenches the fluorescence via intermolecular PET between the photoproduct and the fluorophore within **16**. In other words, the fluorescence in the illuminated regions undergoes an 'off-on-off' process as a function of writing time. Our focus is on the edges between illuminated and dark regions because a bright fluorescent border becomes visible here alone. This occurs because H^+ diffuses from the illuminated areas into the unilluminated areas to switch 'on' the fluorescence of **16** which are localized here. Fluorescence is not quenched because the quenching photoproduct is too slow to diffuse across from the illuminated regions during the timescale of the experiment. The sharpness of the visualized edge depends on slowing the H^+ diffusion rate by controlled drying of the paper matrix. Overall, the paper serves as a graphic user interface where large numbers of molecules work together to solve the edge visualization problem.

In order to appreciate what the molecules have achieved in this instance, it is worth considering how semiconductor computers solve the same problem. The object is raster-scanned and sharp discontinuities of light intensity occur each time an edge between illuminated and unilluminated regions is crossed. Contiguous points of these discontinuities are joined together to build lines in the two-dimensional field. A degree of spatial smoothing is also applied. All these operations require a full computer or microprocessor running such an algorithm. This represents integration of logic gates in molecular systems (de Silva *et al*., 1999) at a medium-scale. Molecular biological systems are now regularly achieving this level of integration owing to their inputs and outputs being composed of DNA oligomers which are compatible with each other (Seelig *et al*., 2006). So we see that the inanimate molecular system of **16**, **17** and specified moisture on paper emulates a considerable amount of complex computation, just like living (Tabor *et al*., 2009) or life-like (Chirieleison *et al*., 2013) molecular systems have done.

Computations that we do consciously

The double input-single output logic, XOR, is notable because it produces a 'high' output only if one or the other input is 'high'. This is particularly important to implement at the molecular-scale (Credi *et al*, 1997; de Silva & McClenaghan, 2002) because it allows an approach to arithmetic when run in parallel with the afore-mentioned AND logic (de Silva, 2013; Gregg, 1998; Malvino & Brown, 1993) with compatible inputs. Ca^{2+} and H^+ serve in this capacity for compounds **18** (XOR) and **19** (AND). AND gate **19** follows in the tradition of **3**, by employing an amine receptor to capture H^+ but with an amino acid receptor to capture Ca^{2+} instead of the benzocrown ether. XOR gate **18** is based on a different design where H^+ binding to the pyridine receptor causes a red-shift in the absorption spectrum whereas Ca^{2+} binding to the amino acid receptor causes a blue shift. Fortunately, the correct occupation of both receptors results in cancellation of the two shifts. The transmittance output pattern fits XOR logic. Then, the fluorescence output of the AND gate produces the 'carry' digit, while the transmittance output from the XOR gate gives the 'sum' digit (de Silva & McClenaghan, 2000). We are all introduced to 'sum' and 'carry' digits as children. Calculators and computers achieve manipulations of decimal numbers via the corresponding binary operations inside their semiconductor processors. The basic processor is the half-adder. So, it is important to show that molecules could do the same, at least at the proof-of-principle level. Progress is being made along this line in many laboratories (Margulies *et al*., 2005).

Another example is related to the edge detection described above. Many children perform line drawing and this activity is developed in adulthood by visual artists. Molecules can be persuaded to do the same by exploiting edge detection. Even artists start with edge detection in their eyes before representing the object as an image on their canvas. So we employed objects of arbitrarily complex shapes to produce line images of good fidelity (Ling *et al*., 2015b). The chemical basis was exactly the same as in edge detection, although more attention needs to be paid to avoid interference of diffusion fronts when different edges approach each other in space.

CONCLUSION

Molecules have carried out computations necessary for living ever since life existed. However, these molecules need to be organized and packaged into organelles, cells, tissues and higher-level assemblies. The past three decades have yielded results which show that no particular organization is necessary for synthetic molecules to accomplish basic logic-based computations on their own. Even some human-level computations can be conducted, albeit at low efficiencies. Lessons learned in computer science and engineering have been applied to molecules to show that semiconductors are not essential to accomplish basic logic-based computations. The chemical behaviour of molecules is the bridge between modern semiconductor-based information technology and the ancient information technology called life.

Many hundreds of laboratories spread across several disciplines including chemistry, molecular biology, molecular physics, molecular materials science, physiology and genetics have published on various aspects of logic-based computation. This trend seems set to continue. In particular, intracellular applications are likely to yield answers to many interesting questions about biological complexity.

Fluorescent sensors led the way to molecular logic-based computation and still occupies a prominent place in the field. The unique ability of fluorescence to visualize processes in small spaces (Hell, 2015) will enable the intracellular applications noted above. Following the lead of cases like **2**, fluorescent sensors will probably play a bigger role in serving the analysis needs of medical and environmental science as the years go by.

Conflict of interest

We declare there are no conflicts of interest.

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RESEARCH ARTICLE

Geo-environmental assessment of geochemistry of groundwater and associated human health risks in the dry zone terrain of Sri Lanka

R Chandrajith[1*] and S Diyabalanage[2,3]
[1] *Department of Geology, Faculty of Science, University of Peradeniya, Peradeniya, Sri Lanka.*
[2] *Instrument Centre, Faculty of Applied Sciences, University of Sri Jayewardenepura, Nugegoda, Sri Lanka.*
[3] *Ecosphere Resilience Research Center, Faculty of Applied Sciences, University of Sri Jayewardenepura, Nugegoda, Sri Lanka.*

Abstract: The close association between the geographic distribution of certain diseases and environmental factors is clearly apparent in the tropical terrain of Sri Lanka. Among these diseases, dental and skeletal fluorosis and Chronic Kidney Disease of uncertain aetiology (CKDu) are common problems in the dry zone regions of the country. The quality of drinking water is primarily blamed for the emergence of such diseases. Since 2015, about 1250 groundwater samples from the dry zone region of Sri Lanka were collected and analyzed for their water quality parameters. Excessive ionic concentrations were observed in groundwater in which solute composition is dominated by Na^+, K^+, Ca^{2+}, Mg^{2+}, HCO_3^-, Cl^-, and SO_4^{2-}. The results revealed that the dry zone was highly affected by excessive levels of fluoride and divalent cations. Fluoride content in the study samples varied up to 6.8 mg/L. About 35% and 73% of samples from metamorphic and sedimentary terrains, respectively, were identified as very hard and not palatable. Higher As content is a significant feature in groundwater from the sedimentary terrain in the north and northwest of the island. Excessive nitrate was also noted in highly permeable sandy aquifers in the coastal belt. Results of available water quality data confirm that the groundwater quality of the dry zone is affected by both geogenic and anthropogenic factors, while certain geographically distributed health issues are closely associated with the groundwater geochemistry.

Keywords: Arsenic, CKDu, dental fluorosis, excess fluoride, medical geology, water hardness.

INTRODUCTION

For centuries, it has been known that human health is strongly influenced by the natural environment, in which geological factors play a crucial role in determining the well-being of biota. In this view, the emerging interdisciplinary science of ‘medical geology’ addresses the interaction between natural geological factors and associated health effects on humans and animals (Dissanayake & Chandrajith, 1999; Bunnell, 2004; Dissanayake, 2005). The availability of trace elements and their species in the geological environment varies widely. The availability of such components may have a beneficial impact on human health, but excessive content leads to detrimental health effects. For instance, it is well-known that human health can be adversely affected by exposure to contaminants such as As, Cd, Hg, Pb, and fluoride. Natural geogenic processes and anthropogenic activities contribute contaminants to the environment at different levels (Bundschuh *et al*., 2017). Biotic and abiotic interactions that occur in the geosphere leads to the accumulation and magnification of elements and their species in trophic chains and food webs, resulting in either beneficial or detrimental effects on organisms (Dissanayake, 2005).

Drinking water is perhaps the most vital natural resource, and an estimated half of the world’s population, particularly in developing countries entirely rely on groundwater for drinking (Kløve *et al*., 2014). Over 66% of households in rural Asia-Pacific regions use groundwater for drinking (Carrard *et al*., 2019). Not only that, groundwater is extensively extracted in this region for irrigation purposes. For instance, 38% of agricultural lands in the world are currently irrigated with groundwater

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[*] Corresponding author (rohanac@pdn.ac.lk; https://orcid.org/0000-0003-3072-5240)

(Siebert *et al*., 2010). For all these purposes, the quality of water is extremely vital and the requirement of clean water is one of the fundamental commodities for human life. Hence, monitoring groundwater quality is one of the most important aspects of water resources management and supply of suitable quality water for human consumption. Particularly in developing countries, the majority of the population depends on groundwater that is extracted and consumed without any pre-treatment. Clean water is often scarce in such regions, and hence groundwater quality monitoring is extremely important. As set forth by the United Nations, access to clean water and sanitation (SDG 6) and good health and well-being (SDG 3) are important aspects of the Sustainable Development Goals (SDGs) that need to be achieved by the year 2030 (UN 2015).

There are ample reports on geographically distributed health issues and their close association with the geo-environment, especially relevant to drinking water quality. For instance, high arsenic (As) and fluoride (F^-) in groundwater have been major public health concerns for several decades (Karim, 2000; He *et al*., 2013; Rodríguez-Lado *et al*., 2013; Wen *et al*., 2013). Such health issues are more apparent in countries in the equatorial belt, which are having unique geological and climatological characteristics and are mostly considered as less developed with many socio-economic issues. These tropical regions continuously experience intense rainfalls and prolonged droughts, which facilitate geochemical partitioning, resulting in depletion or enrichment of elements (Dissanayake & Chandrajith, 1999). This might be more severe in the near future due to predicted climate change impacts, and more effects may focus on water quality and water stress in many regions (Kundzewicz *et al*., 2008).

Sri Lanka is an Indian Ocean island with a population of 22 million, of which only about 44% have access to a pipe-borne water supply, while the rest mainly depend on the tube and dug wells (Fan, 2015). With its varied geology, topography, and climate, the quality of water also varies widely. Although the island is small, with only about 65,000 sq km land area, distinct rainfall regimes with different climatic conditions can be identified. From which, the wet zone receives an average annual rainfall of about 2500 mm, while the dry zone receives significantly lower annual precipitation (<1500 mm) (Figure 1). Over 60% of the land area of Sri Lanka belongs to the dry zone where rainfall is restricted to a few months of the year. In this climatic region, most households depend on groundwater for drinking. In some regions, the entire population depends on groundwater for their daily needs. As a country in the tropical belt, a close relationship between human health and the immediate natural environment is more visible in Sri Lanka (Dissanayake & Chandrajith, 1999). In the last few decades, several studies were carried out in Sri Lanka to illustrate the impact of natural geological factors and the distribution of certain chronic diseases, health, and nutrition. Groundwater fluoride and dental and skeletal fluorosis (Dissanayake, 2005; Chandrajith *et al*., 2012; 2020), nitrate in drinking water and related health impacts (Dissanayake & Weerasooriya, 1987), iodine and iodine-deficient disorders (IDD) (Dissanayake & Chandrajith, 1993), and water hardness and occurrence of urinary calculi (Abeywickarama *et al*., 2016) are among many others. Over the last two decades, a new form of Chronic Kidney Disease without any known etiological factors (CKDu) has emerged in the dry zone of Sri Lanka and is also considered to be associated with the quality of drinking water (Chandrajith *et al*., 2011b; Wickramarathna *et al*., 2017; Nanayakkara *et al*., 2019; Balasooriya *et al*., 2020; Liyanage *et al*., 2022). These facts suggest Sri Lanka as an ideal location to investigate the geochemistry of groundwater, which is a very useful tool for delineating regional health issues and identify the geographic distribution of chronic diseases. The aetiology of certain geoenvironment-related diseases can be delineated effectively with the help of an investigation of the geochemical composition of groundwater. Therefore, the need of monitoring the groundwater quality in Sri Lanka is of foremost priority in achieving SDGs by the year 2030. In the dry zone regions of Sri Lanka, over 87% of the community depends on groundwater extracted from shallow dug wells (<10 m) and deep tube wells for their domestic needs (Dissanayake & Chandrajith, 2018). The utilization of groundwater for drinking with no pre-treatment indicates a link between groundwater geochemistry and certain health issues. Therefore, this paper aims to present hydrogeochemical data giving particular attention to the dry zone area where most of the environment-related health issues are widely reported. The improved understanding of the quality of groundwater is important in identifying possible relationships with geographically distributed health issues, and also for water resource management for ensuring the sustainable use of groundwater to achieve the proposed SDGs.

Climate, geology, and groundwater

Sri Lanka has a humid tropical climate with monsoon rainfall and well-demarcated spatial variation. The southwest monsoon brings heavy rains into the southwest wet zone region with over 2500 mm/a precipitation while the northeast monsoon mainly falls in the dry zone with about 1000 mm/a rain. In between these two climatic zones, an intermediate zone is also characterized with 1700 mm/a precipitation while in the dry zone, evaporation exceeds the precipitation (Jayasena *et al*., 2008). Geologically, over 90% of the island of Sri Lanka comprises amphibolite and granulite facies metamorphic rocks, and the groundwater in this terrain is mostly extracted through deep (tube) and shallow wells (Dissanayake & Chandrajith, 2018). Shallow wells are often constructed in the unconsolidated overburden, while deep wells penetrate to fractures, cracks, and fissures in the hard rocks. A marked difference in physicochemical parameters can also be observed between shallow and deep wells, although a seasonal variation of water quality is not apparent (Wickramarathna *et al*., 2017; Senarathne *et al*., 2021). A narrow terrain in the northern and north-western coastal belt is underlain by Miocene limestone sequences that overlay Quaternary deposits, mainly dune sands. In this region, groundwater is mostly extracted by shallow wells penetrated to either karst aquifers or groundwater trapped in perch water lenses in dune sand. The water quality in these sedimentary aquifers is drastically different from groundwater from the metamorphic terrain (Thilakerathne *et al*., 2015).

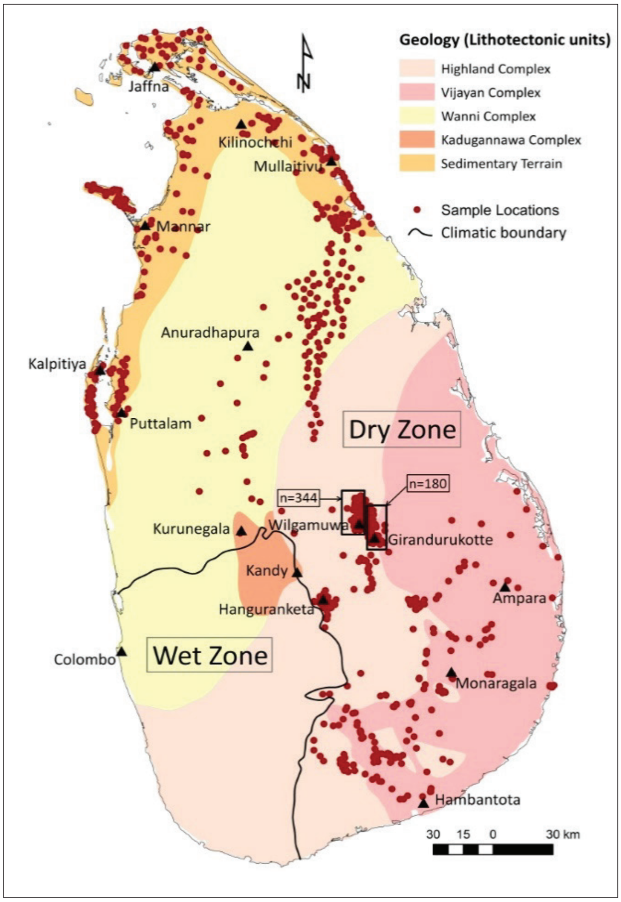

Figure 1: Geological, climatological characteristics of Sri Lanka and sampling locations

METHODOLOGY

Sample collection

Since 2015, extensive investigations on groundwater geochemistry in the dry zone terrain of Sri Lanka have been carried out by the Department of Geology, University of Peradeniya, Sri Lanka. During this period over 1250 groundwater samples were collected which 966 samples were obtained from regolith aquifers in the metamorphic terrain (Figure 1). In this study, primary and secondary data were considered together and analyzed to obtain a comprehensive overview of the water quality in the dry zone of Sri Lanka. Secondary data for this work were obtained from previous studies (Chandrajith *et al*., 2016; Wickramarathna *et al*., 2017; Bandara *et al*., 2018; 2020; Jayathunga *et al*., 2020; Udeshani *et al*., 2020; Senarathne *et al*., 2021; Liyanage *et al*., 2022).

Analytical methods

For the analyses of all water samples, methods described by the American Public Health Association (APHA, 1998) were used. The pH and Electrical Conductivity (EC) were measured using field-portable EC-pH meters. Filtered and unacidified samples were used for anion analysis. For cation analysis, samples were filtered using 0.45 µm nylon filters and subsequently acidified with 1% v/v HNO_3. All samples were kept in the dark at 4 °C until analyses were performed. Alkalinity and hardness were determined by sulphuric acid and EDTA titrimetric methods, respectively. Anions, mainly fluoride, chloride, nitrate, and sulphate contents were measured either by ion chromatography (Thermo Dionex ICS-1100) or using a visible spectrophotometer (Hach 2400). Major cations (Na^+, K^+, Ca^{2+}, Mg^{2+}) were measured by atomic absorption spectrometry (Varian 240FS). In addition, trace metals in groundwater samples were measured by Inductively Coupled Plasma Mass Spectrometry (Thermo Scientific iCAPQ). Detailed procedure and quality control adopted in sample analysis were described in Balasooriya *et al*. (2020). Concentrations below the detection limits were allocated a value of half of the limit of detection (LOD) during statistical interpretations. The analytical precision was determined by calculating the cation-anion balance considering major cations (Na^+, K^+, Ca^{2+}, and Mg^{2+}) and anions ($F^–$, $Cl^–$, $NO_3^–$, $HCO_3^–$, and $SO_4^{2–}$) and it was within 10% in most cases.

RESULTS AND DISCUSSION

Geochemical characteristics of dry zone groundwater

The summary results of the analyzed physicochemical parameters are given in Table 1. The pH of the groundwater in the dry zone region of Sri Lanka varied from slightly acidic to alkaline. In the metamorphic terrain, the median pH was 6.75 (± 0.65); however, a higher median pH (7.42 ± 0.66) was observed in groundwater in the sedimentary terrain. Higher evaporation rates and dissolution of minerals in soils and rocks may cause increased pH in groundwater. The electrical conductivity (EC) varied from 4.94 µS/cm to 5.22 mS/cm in the metamorphic terrain, with a median of 324 µS/cm, implying a higher rate of mineralization. According to Sri Lanka Standards (SLS), an EC of 750 µS/cm is considered as the maximum permissible limit for drinking water (SLS, 2020); 21% of the dry zone samples from the metamorphic terrain exceeded this level. Further, 39% and 20% of samples were categorized into medium salinity (250–750 µS/cm) and high salinity (750–2250 µS/cm) groups, respectively, based on the EC classification of Handa (1969). Interestingly, comparatively higher EC values were reported in the sedimentary terrain that varied from 16.2 µS/cm to 10.9 mS/cm with a median of 1056 µS/cm, while 63% of the samples exceeded the maximum permissible limit for drinking water (Figure 2). Higher amounts of dissolved solids in groundwater from the sedimentary terrain resulted from the dissolution of carbonate rocks and the mixing of saline water (Chandrajith *et al*., 2016; Bandara *et al*., 2018).

The major solute composition of all the dry zone water is dominated by Na^+, K^+, Ca^{2+}, Mg^{2+}, HCO_3^-, Cl^-, and SO_4^{2-}. In most wells, anion abundances followed the sequence $HCO_3^– > Cl^– > SO_4^{2-} > NO_3^-$. Chloride contents in the metamorphic terrain varied from 0.20 to 3100 mg/L with a median of 25.0 mg/L. However, only 3.6% of the samples collected near the coastline exceeded the recommended limit. In contrast, 45% of the samples from the sedimentary terrain showed higher Cl^- levels that are mainly due to seawater intrusions. The median sulphate of groundwater in the metamorphic terrain was 12.2 mg/L, while it was 37.4 mg/L in the sedimentary terrain. Accordingly, 14% of the samples from sedimentary terrain showed higher sulphate contents (>250 mg/L), and exceptionally higher values (>500 mg/L) were mostly observed in the Kalpitiya region. This is mainly due to the extensive application of chemical fertilizer in the region (Jayathunga *et al*., 2020).

Among the investigated anionic constituents, a comparatively higher nitrate content was noted in the dry zone sedimentary terrain at a median of 1.20 mg/L, while it was 0.90 mg/L in the metamorphic terrain. The primary sources of nitrate in groundwater are fertilizer applications, septic tank leachates, and leaching from open waste dumps. Although agriculture is widespread throughout the dry zone terrain, all the values reported in the crystalline rock terrain were well below the recommended limits (50 mg/L as NO_3^-). In contrast, aquifer materials of sedimentary terrain are highly permeable and can easily be contaminated due to anthropogenic activities. This is clear in the reported highest nitrate values, which exceed the maximum permissible level in the Kalpitiya region where extensive fertilizer applications are reported. In addition, as evident from water isotope signatures, irrigation return flows can also increase the nitrate content with the continuous applications of nitrogenous fertilizers in areas that are underlain by sedimentary rocks and sediments (Chandrajith *et al*., 2016; Jayathunga *et al*., 2020).

The hardness of water is one of the main characteristic features of the groundwater in the dry zone terrain of Sri Lanka. The median hardness (as $CaCO_3$ equivalent) reported in the metamorphic terrain was 130 mg/L whereas 286 mg/L was reported in the sedimentary terrain (Table 1). From among samples from the metamorphic terrain, 28% and 19% fell in the moderately hard (61–120 mg/L) and hard (121–180 mg/L) categories, respectively. Nearly 35% of the samples can be categorized as very hard water (>180 mg/L) (Figure 2 and 3). The recommended range for hardness in drinking water is between 100 and 150 mg/L and the maximum desirable limit is 250 mg/L (SLS, 2020). Among all collected samples, 23% exceeded the 250 mg/L limit while in the sedimentary terrain, 73% of the samples were very hard and 59% exceeded the 250 mg/L limit.

Table 1: Major element composition of groundwater in studied wells

Parameter	Metamorphic terrain							Sedimentary terrain							Maximum permissible level	
	Mean	Median	SD	Min	Max	Percentile		Mean	Median	SD	Min	Max	Percentile		Sri Lankan Standards; SLS 614, 2013	WHO Standards; WHO, 2022
						25	75						25	75		
pH	6.74	6.75	0.65	4.49	8.96	6.27	7.18	7.37	7.42	0.66	5.10	9.36	7.10	7.79	6.0 - 8.5	6.5 - 8.5
EC (µS/cm)	492	324	526	4.94	5224	154	666	1720	1056	2020	16.2	10900	462	2145	750	1500
TDS (mg/L)	504	344	533	12.9	5442	156	712	1293	792	1284	69.8	5950	495	1592		
Alkalinity (mg/L)	214	150	203	3.60	1865	76.0	324	214	214	171	1.60	826	39.2	340	200	500
HD (mg/L)	180	130	176	4.00	2210	72.9	234	475	286	634	16.9	5810	157	518	250	300
Anions (mg/L)																
NO_3^-	1.78	0.90	2.63	< 0.02	27.0	0.50	2.20	12.4	1.20	63.4	< 0.02	845	0.50	4.85	50	50
SO_4^{2-}	22.8	12.2	42.9	< 0.05	690	6.00	24.4	126	37.4	254	0.05	2166	14.8	120	250	250
PO_4^{3-}	0.33	0.21	0.58	< 0.01	9.90	0.06	0.37	0.88	0.44	1.58	< 0.01	11.3	0.26	0.81	2	0.1
Cl^-	57.1	25.0	149	< 0.20	3100	13.9	49.8	464	190	708	5.55	4830	64.1	561	250	250
F^-	0.82	0.57	0.83	< 0.02	6.80	0.29	1.08	0.51	0.39	0.52	< 0.02	5.04	0.17	0.68	1	1.5
Major elements (mg/L)																
Na	43.6	24.4	62.9	0.08	654	11.4	46.9	148	61.2	249	6.37	1700	34.4	130	250	200
K	2.83	1.39	4.55	0.12	51.0	0.72	2.90	14.9	6.91	25.1	0.27	267	2.84	18.0	ND	20
Ca	62.1	32.1	113	0.20	1451	16.1	61.8	117	76.9	151	2.96	1164	35.8	141	100	75
Mg	19.3	12.5	26.0	0.05	472	5.65	24.4	33.5	20.0	47.8	1.53	470	9.30	36.5	30	50
Trace elements (µg/L)																
Li	3.27	1.43	5.59	< 0.02	50.3	0.71	3.22	12.6	4.13	20.1	< 0.02	147	1.27	15.8	ND	ND
Al	44.9	9.03	176	< 0.05	2561	4.01	20.1	38.1	9.20	136	0.16	1680	4.71	23.6	200	200
Cr	1.78	0.38	6.30	< 0.01	88.1	0.16	1.04	7.13	0.52	43.2	0.01	599	0.23	1.14	50	50
Mn	145	33.9	337	0.01	4542	6.87	119	126	41.6	254	0.45	2268	7.31	129	100	80
Fe	252	25.1	783	< 0.01	8152	8.72	82.8	463	52.4	3082	0.82	46197	19.4	118	300	300
Co	0.77	0.15	1.91	0.01	17.2	0.06	0.52	1.43	0.27	4.9	0.02	67.7	0.10	1.02	ND	ND
Ni	26.4	1.74	112	0.01	1129	0.90	3.80	7.61	2.26	17.3	0.19	145	1.24	6.57	20	70
Cu	8.54	0.80	36.1	0.01	432	0.40	1.95	9.05	1.50	45.2	0.01	560	0.77	2.96	1000	2000
Zn	115	24.9	1113	0.03	32176	9.75	53.9	88.8	26.4	206	0.35	2332	12.5	79.2	3000	3000
As	0.35	0.20	0.53	< 0.02	8.14	0.10	0.39	3.07	1.01	5.6	0.03	46.9	0.34	3.39	10	10
Se	0.66	0.27	1.27	< 0.02	14.6	0.07	0.69	1.82	0.57	2.8	< 0.02	20.8	0.25	2.11	10	40

Continued -

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Parameter	Metamorphic terrain							Sedimentary terrain							Maximum permissible level	
	Mean	Median	SD	Min	Max	Percentile 25	Percentile 75	Mean	Median	SD	Min	Max	Percentile 25	Percentile 75	Sri Lankan Standards; SLS 614, 2013	WHO Standards; WHO, 2022
Rb	4.58	1.21	15.9	0.16	220	0.65	3.10	11.8	9.75	12.1	0.34	76.1	3.19	16.8	ND	ND
Sr	353	226	403	4.30	4016	118	443	1339	765	2048	28.4	21000	326	1459	ND	ND
Cd	0.29	0.03	1.96	< 0.01	43.0	0.01	0.09	0.11	0.07	0.2	< 0.01	3.44	0.03	0.10	3	3
Ba	140	83.1	161	1.40	1477	46.5	169	146	96.4	153	2.90	1124	42.1	190	ND	700
Pb	0.83	0.15	3.51	< 0.01	65.4	0.06	0.42	0.94	0.12	2.9	< 0.01	37.2	0.07	0.79	10	10

EC- electrical conductivity; TDS- total dissolved solids; HD- total hardness; ND- not defined

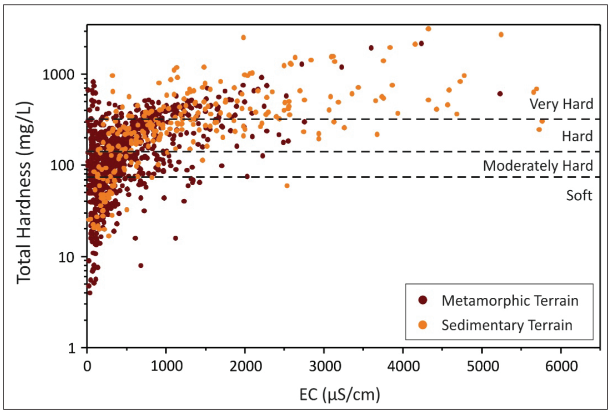

Figure 2: Relationship between water hardness and electrical conductivity (EC) of groundwater in the study area

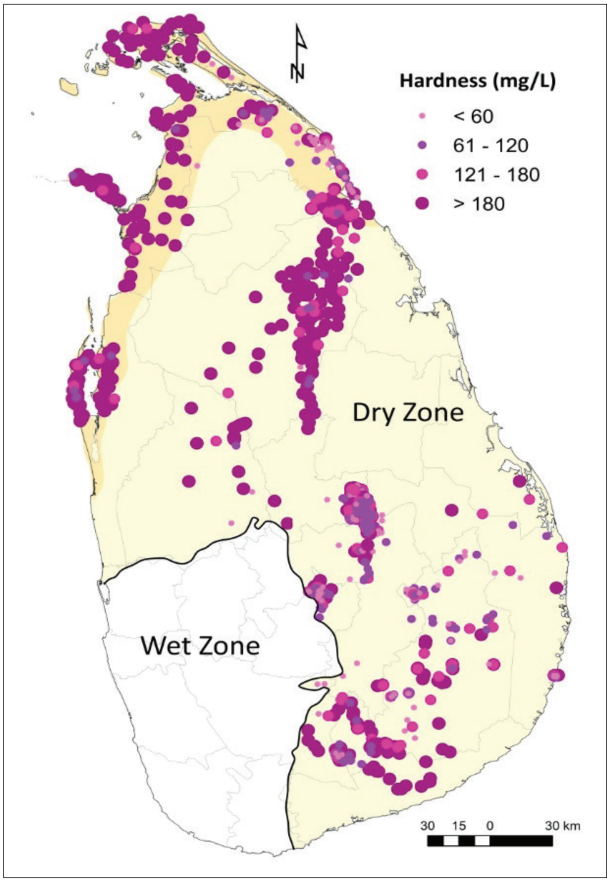

Figure 3: Distribution of the groundwater hardness in the dry zone of Sri Lanka

Fluoride is an important hydrogeochemical parameter that enters the human body primarily from drinking water, and hence the geochemistry of fluoride in a region is of extreme importance. The dry zone of Sri Lanka is well-known for a higher content of fluoride in drinking water, with reported health issues of dental and skeletal fluorosis (Dissanayake & Chandrajith, 1999; Dissanayake, 2005; Ranasinghe *et al*., 2019b). The fluoride content in hard rock terrain varied from < 0.01 to 6.80 mg/L with a median of 0.57 mg/L, of which 35% exceeded the recommended value of 0.8 mg/L for tropical countries (WHO, 2004) while 15% of those samples exceeded the 1.5 mg/L limit (WHO, 2011). In the sedimentary terrain, the fluoride content varied from < 0.01 to 5.04 mg/L with a median of 0.39 mg/L, of which 17% exceeded the 0.8 mg/L limit (Figure 4). However, Warnakulasuriya *et al*., (1992) inferred that dental fluorosis is common among school children who drink water over 0.8 mg/L of fluorides.

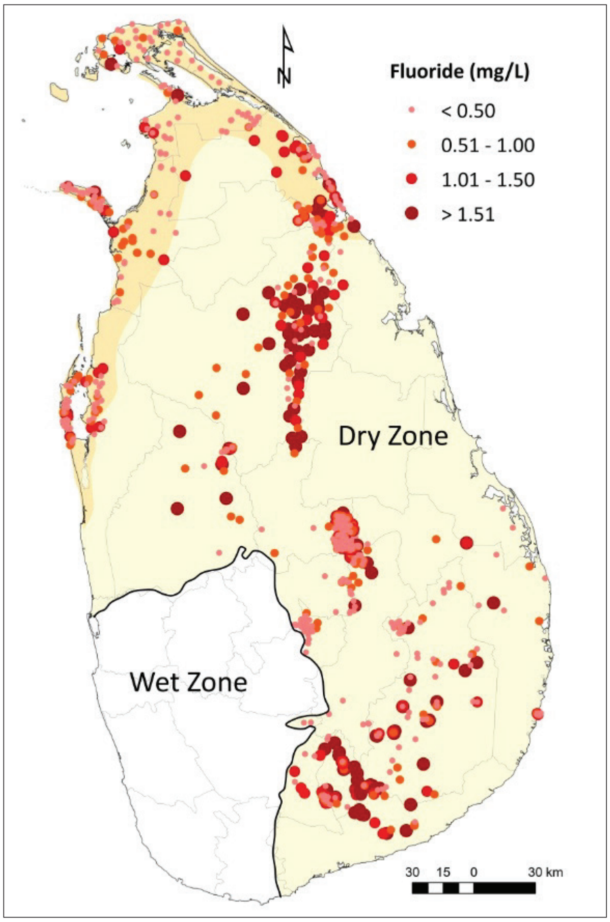

Figure 4: Distribution of the groundwater fluoride in the dry zone of Sri Lanka

The geochemical distribution pattern of fluoride in Sri Lanka depends on the source of groundwater, mineralogical composition of rocks and soils, and the climatic pattern (Dissanayake & Chandrajith, 1999; Chandrajith *et al*., 2012). In contrast, groundwater in the wet zone showed remarkably lower fluoride content. Higher rainfall in the wet zone results in rapid leaching of fluoride ions from rocks and soils; the fluoride is then washed away (Dissanayake, 1996). The rocks in the metamorphic terrain comprise fluoride-bearing minerals, mainly apatite, micas, and amphibolites, and the ion exchange with hydroxyl groups (OH^-) eventually increases the fluoride (F^-) leaching into groundwater. Higher pH, the presence of bicarbonates, and the availability of alkali and alkaline earth elements also contribute to the increased mobility of fluoride into groundwater (Saxena & Ahmed, 2003; Jacks *et al*., 2005). Additionally, higher evaporation under a tropical climate may enrich the fluoride content in groundwater (Dissanayake, 1996; Mukherjee & Singh, 2020). Although fluoride-bearing minerals are absent in the terrain underlain by the Recent deposits and Miocene limestone sequences, the application of phosphate-bearing fertilizer in these regions may increase the fluoride content in groundwater. Phosphate minerals are a well-known host for fluoride (Edmunds & Smedley, 2013).

Among the major cations, Na^+, Ca^{2+}, and Mg^{2+} are dominant in groundwater from the dry zone region. In the metamorphic terrain, the Na^+/Cl^- molar ratio was found to be >1, which implies the release of Na^+ during the process of silicate weathering (Figure 5). The ion exchange of Na^+ and K^+ in rocks with Ca^{2+} and Mg^{2+} in water is evident in this process (Li *et al*., 2008). The presence of Ca^{2+} and Mg^{2+} ions that contribute to the water hardness are considered to be an important parameter as it may possibly influence health issues such as the occurrence of urinary calculi (Abeywickarama *et al*., 2016). In the metamorphic terrain, 11% and 18% of the samples exceeded the maximum permissible levels for Ca^{2+} and Mg^{2+}, respectively. In the case of limestone terrain, 38% of the samples contained higher Ca^{2+} levels, exceeding the 100 mg/L limit, while 33% showed Mg^{2+} exceeding the 30 mg/L limit. Significant correlation of Ca^{2+} and Mg^{2+} with HCO_3^- signifies the dissolution of Ca-Mg bearing minerals in the metamorphic terrain. The interrelationship of ion ratios and salinity of groundwater in the Gibbs diagram (Gibbs, 1970) showed that the geochemistry of most of the dry zone water is governed by rock water interaction and silicate weathering and carbonate dissolution as key processes (Figure 6).

Weathering of the underlying rock and associated ion exchange processes are the key processes behind the major ionic constituents in groundwater. Some of the dry zone water, particularly from the sedimentary terrain, is influenced by evaporation and mixing of saline water, in agreement with the locations and prevailing dry climate.

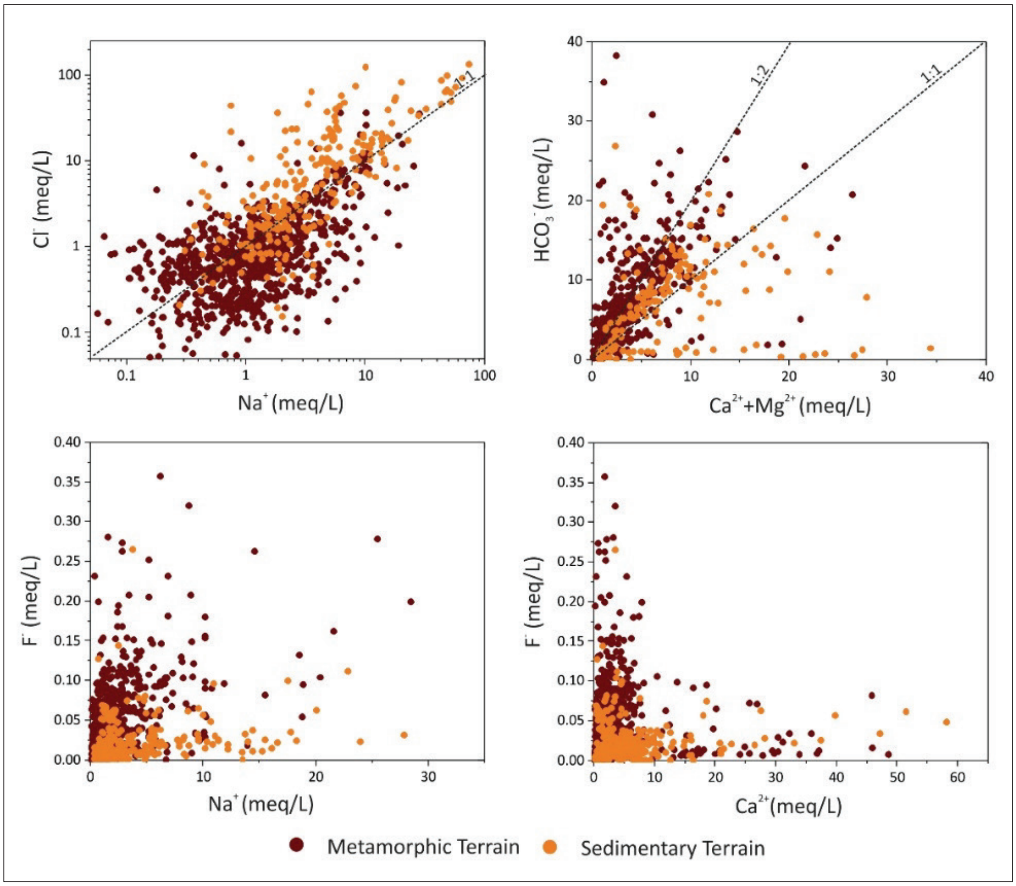

Figure 5: Ion scatter plots of major parameters in groundwater

Trace elements in groundwater

From a health perspective, some of the trace elements can cause toxic impacts on humans and animals even at very low concentrations. This situation is more problematic in areas where the community relies mostly on groundwater for their drinking purposes. The content of most toxic trace elements in groundwater was lower than the Sri Lankan drinking water quality standards and also the standards prescribed by WHO (2011) and USEPA (2012). Among the studied trace elements, Sr and Ba contents dominated in groundwater compared to other trace elements in both geological terrains (Table 1). The median content of total iron (Fe) in groundwater from metamorphic and sedimentary terrains was 25.1 µg/L and 52.4 µg/L, respectively, and 14% of the studied wells from each terrain exceeded the desired levels of iron in drinking water (0.3 mg/L). From among the top ten trace elements of major public concern, arsenic, cadmium, and lead in groundwater have become a global concern due to their high toxicity, persistence, and bio-accumulative nature (WHO, 2021). However, none of the samples from the metamorphic terrain exceeded the permissible limit for arsenic (10 µg/L). However, 6% of the samples from the sedimentary terrain exceeded the 10 µg/L limit; the majority of these samples were collected from the Mannar island. In most samples, Cd levels were at or below the detection limit of the ICP-MS (0.01 µg/L).

The Pb content in the majority of samples from both metamorphic and sedimentary terrains was well below the permissible limit of 10 μg/L, however, 13 samples from the metamorphic terrain and 2 from the sedimentary terrain exceeded the 10 μg/L limit. Selenium (Se) which is also known as an essential trace nutrient, was found to vary from < 0.02 μg/L to 14.6 μg/L in the metamorphic terrain and 0.02 to 20.8 μg/L in sedimentary terrain with median values of 0.27 μg/L and 0.57 μg/L, respectively.

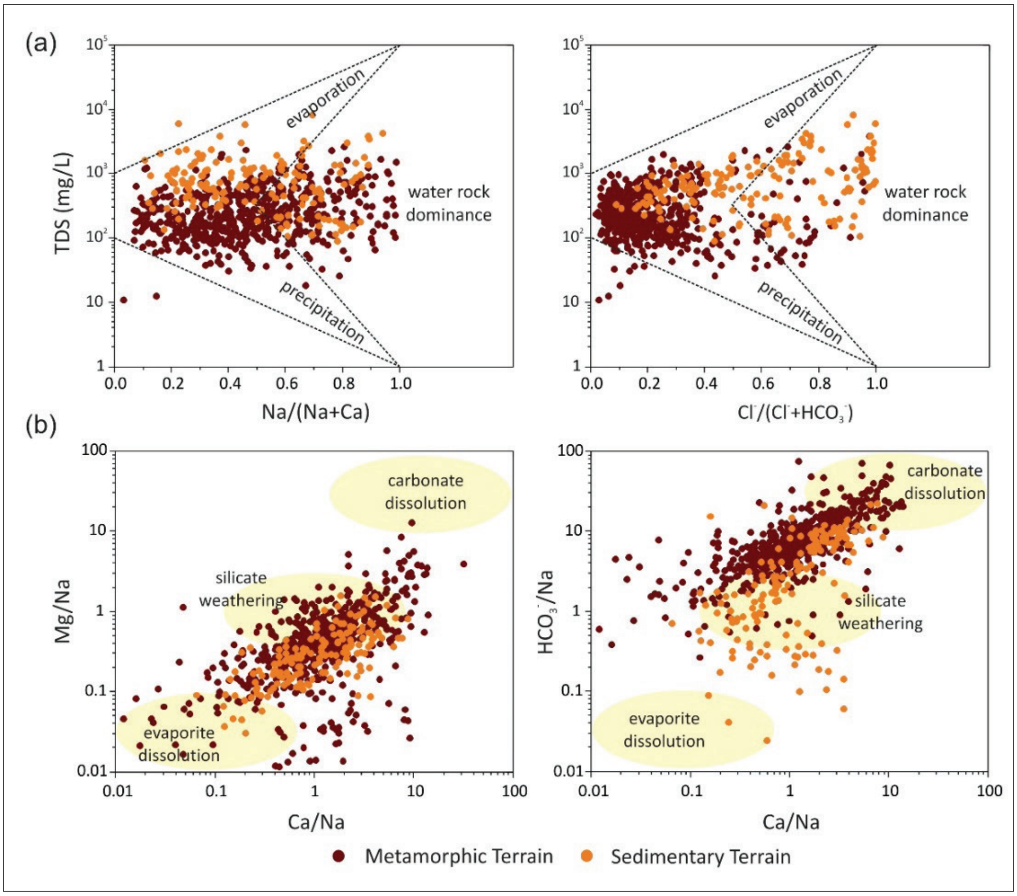

Figure 6: (a) Gibbs plot and (b) bivariate plot explaining the geochemical processes

Possible influence on health issues

Fluoride

It is well known that the lack of fluoride in drinking water causes tooth decay. On the other hand, high concentrations of fluoride can also lead to detrimental health issues. Dental fluorosis (mottling of teeth) and skeletal fluorosis (crippling of bones) can occur when the drinking water fluoride levels exceed 1.5 mg/L and 4.0 mg/L, respectively (WHO, 2011). However, these levels do not apply to tropical terrain where people consume more water due to local environmental conditions. For instance, health effects of fluoride have been observed even at low-level exposures particularly in tropical regions (Ghosh *et al*., 2013). Therefore, the limit of 0.8 mg/L has been defined for tropical climates by the WHO (2004). Although fluoride is beneficial in preventing dental caries by re-mineralization of enamel, it is not considered as an essential nutrient for human well-being, as it is not found in natural human metabolic pathways and no deficiency diseases have ever been reported (Peckham & Awofeso, 2014).

In the dry zone of Sri Lanka, excessive fluoride content in groundwater has been identified as a serious issue, as it compromises the oral health of children (Figure 7). Nearly 80% of the children from dry zone areas are affected by dental fluorosis (Van Der Hoek *et al*., 2003; Rajapakse *et al*., 2017). Moreover, some early studies provided evidence for severe forms of dental fluorosis (Ekanayake & van der Hoek, 2002). Ranasinghe *et al*. (2019b) revealed that 11.2% of the dry zone population (2.2 million) is at risk of potential health issues posed by high fluoride ingestion (>1.0 mg/L) via drinking water. The results of the present study also confirm the severity of the issue, as it revealed that 31% of the groundwater samples showed excess fluoride levels. Long-term consumption of such water may enhance the prevalence of dental fluorosis and also may cause an array of health issues. However, a heterogeneous distribution of fluoride content is depicted in the studied wells in which wells with low fluoride (< 0.5 mg/L) were located close to wells with higher fluoride (Figure 4). Ranasinghe *et al*. (2019b) identified that the minimum distance between a well with high fluoride (> 4.0 mg/L) and low fluoride (< 1.5 mg/L) is closer to 42 m. Since the effect of seasonal variation of fluoride in dry zone wells is negligible (Wickramarathna *et al*., 2017), the wells with safe fluoride can be used as sources of a drinking water supply to the inhabitant population.

Figure 7: A typical case of dental fluorosis in the dry zone of Sri Lanka

The emerging incidence of chronic kidney disease with undetermined origin (CKDu) is one of the main health issues that contribute significantly to high morbidity and mortality rates among dry-zone farming communities in Sri Lanka (Vlahos *et al*., 2019; Fernando *et al*., 2020a). Thousands of deaths have been reported in the dry zone of Sri Lanka during the past 2-3 decades due to CKDu and other kidney-related health problems (Ranasinghe *et al*., 2019a). The prevalence of CKDu between 15.1 and 22.9% has been observed among the residents in affected regions (Jayatilake *et al*., 2013; Kafle *et al*., 2019; Vlahos *et al*., 2019). The geographic distribution of disease shows an uneven patchy pattern in which CKDu villages are located adjacent to non-CKDu villages (Balasooriya *et al*., 2020). The inferior quality of the drinking water consumed by the affected community is the most suspected cause for the disease (Chandrajith *et al*., 2011b; Wickramarathna *et al*., 2017; Nanayakkara *et al*., 2019; Balasooriya *et al*., 2020; Liyanage *et al*., 2022). To a certain extent, the areas with CKDu overlap with the areas with high fluoride levels (Figure 4 and 8) and the excess fluoride intake

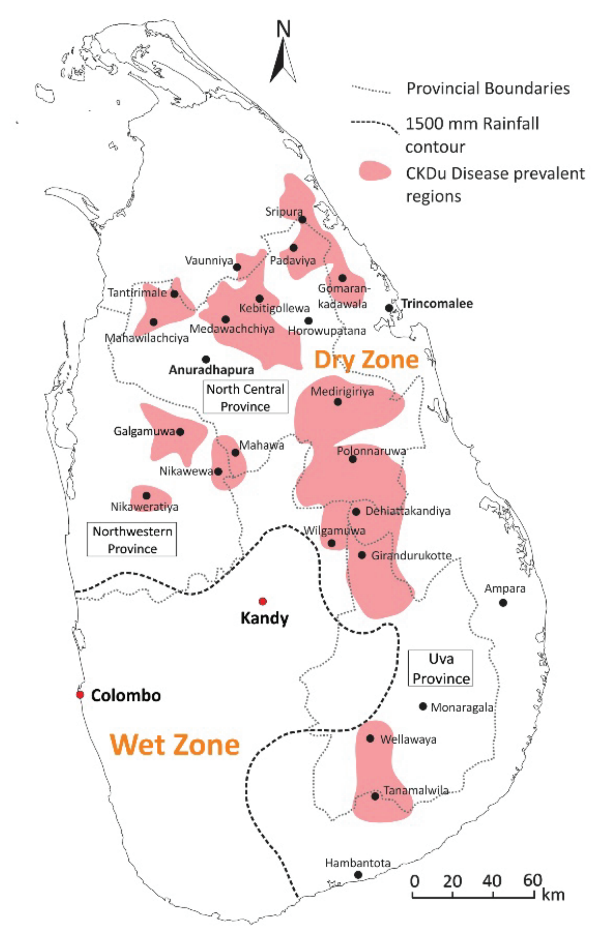

Figure 8: Identified CKDu hotspots of the dry zone of Sri Lanka

can lead to kidney damage (Dissanayake & Chandrajith, 2017; 2019). Fluoride excretion from the body makes kidneys a target organ for fluoride accumulation and the nephrotoxic nature of fluoride has been proven in previous studies (Chattopadhyay *et al*., 2011; Niu *et al*., 2016). It was also reported that serum fluoride levels are higher in CKDu patients compared to the control group in the same area (Fernando *et al*., 2020b). Molecular-level damage can occur in kidneys due to long-term exposure to fluoride, and impaired kidneys may retain more fluoride, which may enhance the damage (Perera *et al*., 2021). When considering the threshold value for fluoride defined for the dry zone of Sri Lanka (0.6 mg/L), 48% of the samples from metamorphic terrain showed higher concentrations, alone with a high hardness that is believed to behave synergistically to damage kidneys (Dissanayake & Chandrajith, 2019; Perera *et al*., 2021). Although the water hardness in the sedimentary terrain, where limestone is predominant, is higher, fluoride levels are much lower in many instances, while CKDu is not reported in these areas.

Nitrates

Although nitrate-related health issues are not investigated in depth in Sri Lanka, a significant correlation between nitrate concentration in groundwater and various types of human cancer such as stomach, small intestine, oesophagus, and liver cancers, total malignant incidence, and benign tumors was reported (Dissanayake & Weerasooriya, 1987). However, the levels of nitrate in groundwater are vitally important as most of the dry zone communities rely on groundwater as their drinking water source. Since rice and vegetable farming is widespread, extensive application of nitrogenous fertilizer can induce the levels of nitrate in the dry zone area. The nitrate derived from fertilizer is readily soluble and can easily leach from the soil. Particularly, the use of nitrogenous fertilizers such as urea $[CO(NH_2)_2]$, ammonium sulphate $[(NH_4)_2SO_4]$, and ammonium nitrate (NH_4NO_3) and mixing of groundwater with irrigated return flows can lead to extreme nitrate levels (Jayathunga *et al*., 2020). As depicted in the Kalpitiya region, the effect of fertilizer on higher nitrate levels may visible over a large area, with up to 846 mg/L nitrate-N in some wells, with a mean of 78 mg/L (n = 43). In addition, the spot-wise elevated values reported in certain other locations, such as the Kalpitiya and Jaffna peninsulas, may also be associated with the leaching of contaminants from pit latrines and waste dumps.

Water hardness

Water hardness (Ca^{2+} and Mg^{2+}) is one of the important parameters that have a strong correlation with the geogenic environment. The majority of the groundwater in the dry zone of Sri Lanka belongs to the moderately hard and hard categories (Figure 2 and 3). Health implications related to the water hardness in Sri Lanka are limited except for the incidences of kidney stones in discrete locations in Sri Lanka (Abeywickarama *et al*., 2016). However, the hardness of water and the dominance of its constituents have been suggested as a possible cause of CKDu. In the dry zone of Sri Lanka, specifically in identified CKDu hot spots, the reported hardness was comparatively higher than the non-CKDu areas (Balasooriya *et al*., 2020; Liyanage *et al*., 2022) (Table 2). The high fluoride content associated with high hardness is a significant hydrogeochemical feature in all CKDu regions of Sri Lanka. Other divalent cations such as Sr^{2+} and Ba^{2+} were also found in higher concentrations in groundwater from the dry zone regions.

Compared to Na^+ activity, Ca^{2+} activity is more prominent in CKDu regions. It has been suggested that lower Na^+/Ca^{2+} ratios in groundwater with elevated fluoride levels could exert negative effects on kidney cells (Chandrajith *et al*., 2011a). A recent study revealed a possible influence of Mg^{2+} on CKDu progression (Liyanage *et al*., 2022). It was suggested that the presence of excess fluoride in hard water with increased Mg^{2+} ions possibly triggers the progression of kidney damage. With the availability of constituent ions, the pairing of fluoride and Mg^{2+} in water may form the $(MgF)^+$ complex, which is nephrotoxic, as indicated by considering the Gibbs free energy calculations for ion pair formation and consideration of Hofmeister-type action of anions (Dharma-wardana. 2018). The formed $(MgF)^+$ complex may disrupt the structure and functionality of proteins by breaking apart and being retained on protein surfaces (Dharma-wardana *et al*., 2015). As such, the prolonged consumption of Mg^{2+} rich hard water with fluoride may synergistically influence kidney damage and/or disease progression. Histopathological investigations on the kidney tissues of laboratory mice, treated with high fluoride water for a long period have revealed acute tubular injury (Wasana *et al*., 2017; Perera *et al*., 2021). Even though the initial damage for CKDu may be caused by some other factors, drinking water with excess fluoride and Mg-hardness could enhance the damage further leading to end-stage renal failure. Although higher hardness is evident in sedimentary terrain, low fluoride content does not lead to chronic renal failures.

Table 2: Concentrations of major constituents of groundwater from identified CKDu hotspot regions

Identified CKDu hotspot region		Parameter							Reference
		HD (mg/L)	F^- (mg/L)	Na (mg/L)	Ca (mg/L)	Mg (mg/L)	As (µg/L)	Cd (µg/L)	
Girandurukotte (n = 180)									
	Min	5.64	0.01	0.08	2.07	0.05	< 0.02	< 0.01	Balasooriya *et al*., 2020
	Max	516	5.20	157	394	76.2	2.54	43.0	Wickramarathna *et al*., 2017
	Mean	135	0.74	24.7	31.8	11.2	0.24	0.94	Present study
	Median	107	0.54	18.5	19.5	7.17	0.16	0.02	
Wilgamuwa (n = 344)									
	Min	8.00	0.01	1.31	3.70	0.26	0.02	< 0.01	Wickramarathna *et al*., 2017
	Max	830	4.40	495	1451	472	4.80	11.38	Present study
	Mean	158	0.71	35.3	94.6	19.6	0.47	0.20	
	Median	124	0.60	24.4	31.6	13.5	0.28	0.03	
Nikawewa (n = 11)									
	Min	236	0.16	14.6	13.8	3.06	0.05	< 0.01	Wickramarathna *et al*., 2017
	Max	628	3.44	157	152	74.3	0.30	0.44	
	Mean	397	1.03	64.5	71.1	35.1	0.15	0.11	
	Median	426	0.79	43.9	36.4	35.3	0.15	0.08	
Moneragala (n = 30)									
	Min	16.6	0.28	4.6	12.3	4.23	0.06	< 0.01	Liyanage *et al*., 2022
	Max	162	6.80	204	131	44.2	0.62	0.12	
	Mean	82.4	2.02	35.0	57.7	24.8	0.24	0.03	
	Median	81.8	1.70	22.7	53.1	27.1	0.17	0.03	
Medawachchiya / Medirigiriya (n = 97)									
	Min	NA	0.08	0.75	3.76	0.15	< 1.0	< 0.14	Levine *et al*., 2016
	Max	NA	2.05	156	161	58.4	1.55	0.17	
	Mean	NA	0.60	39.8	55.4	20.5	NA	NA	
	Median	NA	NA	NA	NA	NA	NA	NA	
Padaviya (n = 34)									
	Min	70	0.02	2.09	2.35	2.08	NA	NA	Chandrajith *et al*., 2011
	Max	816	1.33	188	113	41.6	NA	NA	
	Mean	443	0.62	58.3	35.0	20.1	NA	NA	
	Median	NA	NA	NA	NA	NA	NA	NA	

HD: total hardness NA: not available

In addition to that, an inverse relationship between mortality with cardiovascular disease and water hardness has been suggested in earlier studies. A significant risk level of cardiovascular disease has been observed among the population who consume soft water (Knezović *et al*., 2014). Particularly, increased Mg levels in drinking water are considered cardio-protective and may reduce the risk of mortality through cardiovascular disease. As such, water with higher Mg levels may be beneficial to populations that have insufficient Mg intake (Monarca *et al*., 2006; Leurs *et al*., 2010).

Arsenic

Arsenic contamination in groundwater around the world, together with serious associated health problems, has been known for decades and nearly 108 countries are now known to be affected (Shaji *et al*., 2021). The inorganic form of arsenic is extremely toxic and can cause both acute and chronic effects. Skin lesions and pigmentation, gangrene and malignant neoplasm, and hard patches on the palms and soles of the feet are the common symptoms of As poisoning (Smedley & Kinniburg, 2002). Even at lower exposure levels, like 5 µg/L via drinking water, it can cause skin manifestations, while chronic exposure can affect kidneys, liver, and lungs (Yoshida *et al*., 2004).

Elemental arsenic occurs in association with sulphide minerals, iron oxides, and clays (Bhattacharyya *et al*., 2003). Leaching from As-bearing minerals in aquifer material is the main source of inorganic As in groundwater and its mobility is governed by geological, climatological, and geochemical characteristics such as pH and redox conditions. In the Sri Lankan context, arsenic content in groundwater has gained wider attention, as it has been suggested as a possible causative factor for the emergence of CKDu (Jayasumana *et al*., 2014). However, many previous studies have reported low availability of arsenic in groundwater in the metamorphic terrain (Wickramarathna *et al*., 2017; Nanayakkara *et al*., 2019; Liyanage *et al*., 2022). This is further confirmed by the available water quality data, in which none of the samples from metamorphic terrain, particularly from the CKDu areas, exceeded the maximum permissible limit for As. A study on keratinized matrices from patients with chronic kidney diseases of uncertain aetiology (CKDu), has also revealed that CKDu patients were not exposed to toxic levels of As (Diyabalanage *et al*., 2017). Therefore, it is quite unlikely that As is contributing to the onset of CKDu or the disease progression. However, in contrast to metamorphic terrain, higher As levels have been reported in the sedimentary terrain, particularly in Mannar and Kalpitiya regions. It has been revealed that As occurs as coatings in mineral grains in silty clay and sandy layers (Acharyya & Shah, 2007; Amarathunga *et al*., 2019). The As adsorbed to Fe-oxyanions in aquifer material may get mobilized with the pH at a near-neutral level and facilitated by reductive dissolution processes (Amarathunga *et al*., 2019). No data on associated health effects in these regions are yet available; hence further investigations are needed.

CONCLUSIONS

Identifying and quantifying the influence of the geogenic environment on human health is quite challenging. The synergistic or antagonistic activity of elements, and long-term exposure to them, may pose a risk of toxicity. The hydrogeochemical data obtained from the dry zone of Sri Lanka supports improved understanding of the possible geo-environmental influences over the prevailing health issues and draws attention to the critical state faced by the dry zone community. Higher contents of dissolved solids were observed and identified as a typical feature of the dry zone groundwater. Among many others, excess fluoride, hardness, and arsenic were identified as alarming issues. Considerable numbers of water sources were found to be rich in fluoride, mainly due to the underlying geology and the hydrodynamic nature of the aquifer system. Hard water with excess fluoride has been identified as a significant feature in CKDu affected regions, hence the prolonged consumption of such water could affect kidneys. The groundwater in certain sedimentary terrains has been identified as highly vulnerable to nitrate pollution. Although the toxic trace elements such as As, Cd, and Pb were mostly found below the WHO permissible range, elevated arsenic levels were found in the sedimentary terrain. Providing safe drinking water to the community is one of the important aspects defined under the sustainable development goals. However, the current state of the groundwater quality in the dry zone is quite alarming and assessment of and monitoring its chemical composition and pollution levels are essential requirements to provide safe water to the community.

Availability of data and materials

The dataset used in this paper is available with the corresponding author upon a reasonable request.

Competing interests

The authors declare that they have no competing interests.

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REVIEW

Microbiome: diversity, distribution, and potential role in sustainable crop production

WGD Fernando* **and A Dolatabadian**
Department of Plant Science, University of Manitoba, Winnipeg, Manitoba R3T 2N2, Canada.

Summary: From microbiomes in cheese to soil, many organisms provide enhancement, enrichment, production, and some degradation to the niche they occupy. Although microbial diversity and microbiomes have been known for centuries, it has become a trendy area to investigate with the advent of omics approaches where a vast amount of information can be gathered about the nature and befits of these microorganisms. This information is crucial given the world's population rise. According to FAO, feeding the world's 9.1 billion people in 2050 will necessitate a 70 percent increase in overall food production. Five steps have been offered to fulfil this increasing global food supply-demand, including decreasing agriculture's footprint, growing more on existing farms, discovering ways to use resources efficiently, modifying our diets, and reducing waste. It is essential to focus on management practices that influence soil health, sustainable productivity, and inorganic inputs to reduce agriculture's footprint. Conventional agriculture has increased agricultural production on a large scale; however, overuse of chemicals has led to food contamination, negative environmental consequences, and disease resistance, all of which have important implications for human health and food security. Microbiome research makes it possible to simultaneously reduce these environmental impacts and steadily increase the quantity and quality of agricultural products. Plant microbiome research focuses on defining the structure and activities of microbial communities in various plant-associated habitats and establishing a relationship between specific microbial taxa and plant performance. The microbes associated with plants in the plant microbiome can provide a variety of advantages, including plant growth promotion, soil fertility and control of pests and plant pathogens. Traditional approaches can only examine a small percentage of the vast unexplored soil microbial world because of their biased detection of microbial genetics and functional diversity through culture methods. Today, with the advent of high-throughput sequencing techniques and a variety of "omics" approaches, researchers can now identify microbiome structure and dynamics along with host interactions on an extraordinary level. These approaches will be valuable in establishing the relationship between the structure and function of the soil microbial community and gaining a better understanding of environmental and ecological processes, with a focus on plant-microbe ecosystems.

Keywords: Crop improvement, genomics, microbiome, pathogen, sustainable agriculture.

INTRODUCTION

Behind every good cheese is an excellent microbiome (Gobbetti *et al*., 2018). Cheese is a dynamic ecosystem providing an ideal environment for microorganisms and serves as a model for the behaviour of microbes in complex communities (Wolfe & Dutton, 2013). From factory to field, as cheese microbes (both beneficial and detrimental) contribute to the final flavour, smell, texture, and colour of cheese, soil microbes play a critical role in growing healthy crops and increasing plant yield. The malting industry is another example explaining the relationships between the microbiome and the food industry. Malt quality is highly dependent on the quality of barley grain. Fusarium head blight caused by *Fusarium* spp. is a devastating disease of barley that negatively impacts the malt's quality. The malting industry faces unique challenges with *Fusarium* that are not experienced in other industries. The malting process creates conditions that favour diverse pathogen growth, including *Fusarium* and production of mycotoxins.

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* Corresponding author (Dilantha.fernando@umanitoba.ca; https://orcid.org/0000-0002-2839-1539)

Though *Fusarium* species are central to several quality issues in the malting industry, they are only one component of the microbiome associated with barley and malt. The malt microbiome is the entire community of microorganisms present during the malting process. This community is complex and dynamic, and it is expected that *Fusarium*-related quality issues only manifest themselves when *Fusarium* is competitively dominant within the malt microbiome. Depending on the nature of interactions and the abundance of the microorganisms present in the malt and barley, their effects may be beneficial or detrimental for the process and the final malt quality. Thus, characterization of the microflora during malting and their interactions with each other is an essential step to developing management strategies that will improve malt quality. The diverse nature of microbiomes is given in this paragraph, so the reader appreciates the diversity, complexity and activity microbiomes carry. In the following parts of this review, we will focus on particularly of the soil, the plant, the seed and their microbiomes. These microbiomes dictate how agricultural production systems operate. The operation of a farming system is also dependent on the grower, growers' inputs and all environmental conditions during plant growth. They are so intertwined, it is at times difficult to completely understand what would be the result if one of those parameters changed.

Being one of the most heterogeneous and diverse habitats on the planet, soil hosts the dynamic environments for billions of microorganisms such as bacteria, fungi, actinomycetes, protists and microorganisms such as nematodes, worms, insects, reptiles, and mammals (Bardgett & van der Putten, 2014). Thus, the soil is a biodiversity reservoir, an essential part of our current ecosystem. The conservation of soil biodiversity is critical for both the environment and agricultural industries (Yang *et al*., 2020), as the loss of soil biodiversity can have a devastating impact on its productivity (Olson *et al*., 2017).

The term 'microbiome', to signify the range of ecological community of microorganisms that may be commensal, symbiotic, or pathogenic found in and on all multicellular organisms, including plants, was first coined by Joshua Lederberg (Lederberg & McCray, 2001). Microbiome refers to the entire habitat, including the microorganisms (bacteria, archaea, lower and higher eukaryotes, and viruses), their genomes (i.e., genes), and the surrounding environmental conditions (Marchesi & Ravel, 2015). On the other hand, 'microbiota' refers to the assemblage of microorganisms found within a specific environment. The microbes associated with plants can be classified into epiphytes or epiphytic that exist on the plant surfaces, endophytes or endophytic that are present within the plant's tissue, whether roots or shoots (endosphere), phyllospheric that live on leaves surfaces (phyllosphere), and rhizospheric that inhabits into the soil adjacent to the roots (Rhizosphere) (Berg *et al.*, 2014; Mueller & Sachs, 2015). The term 'rhizosphere' is also defined as the narrow soil region surrounding the roots and is directly influenced by microbes and root secretions (Vishwakarma *et al*., 2020).

Agricultural products based on the microbiota are among the fastest-growing sectors, with a compound annual growth rate of 15–18% and a predicted value of over 10 billion USD by 2025 (Dunham Trimmer, 2017). Therefore, exploiting soil microorganisms associated with crop plants can be considered one of the most promising long-term solutions to the fundamental issues of ensuring food security while preserving a healthy agroecosystem (Singh & Trivedi 2017; Singh *et al*., 2020).

Soil microbiome plays vital roles in the dynamics of plant community and has enormous impacts on soil physicochemical characteristics (Lehmann *et al*., 2017; Sui *et al*., 2021), nutrients bioavailability, mainly nitrogen or phosphorus (Bartlett *et al*., 2007; Lurthy *et al*., 2021), carbon cycle (Jansson & Hofmockel, 2020) and plants physiological processes (Li *et al*., 2018; Saleem *et al*., 2019; Abdul Rahman *et al*., 2021) such that this intricate microbiome is also known as the plant's secondary genome (Berendsen *et al*., 2012). At the same time, hosts' genotypic attributes, growth stage, soil characteristics and environmental conditions affect the dynamics of the microbiome (Rossmann *et al*., 2017).

Although only a fraction of the soil microbiome is known, it is still safe to say they play an essential role in agriculture, food production and climate regulation (Babalola *et al*., 2020; Jansson & Hofmockel, 2020). Thus far, plant-microbe interactions studies have focused mainly on the impacts of a small number of different microbial species, which often reveal contradictory results when utilized in the field (Gadhave *et al*., 2016; Timmusk *et al*., 2017), whilst an alternate approach is to emphasis on the whole microbiome (Pineda *et al*., 2019). For example, Busby *et al*. (2017) stated that introducing more complex soil communities, rather than single species, is essential to maintain a constant high level of crop protection. Thus, microbiome research is important to know the microbial ecology in the soil to support ecosystem functions (Sergaki *et al*., 2018).

In the past decades, molecular methods have been combined with traditional microbiology approaches to offer new intuitions into soil microbial ecology. In recent years, the development of tools in molecular biology, such as sequencing technologies, has provided researchers with more complete and consistent information about microbial ecology (Lucaciu *et al*., 2019). For instance, high-throughput sequencing of marker gene amplicons is used to illuminate microbial communities' composition, organization, and spatial distribution in the environment and is increasingly used in microbiome research (Knief, 2014). Moreover, metagenomic approaches can be accompanied by other omics data, such as transcriptomics, proteomics, and metabolomics, to estimate microorganisms' structure and functional potential in soil (Nesme *et al*., 2014; Lucaciu *et al*., 2019). Hence, microbiome engineering might be an alternate method of understanding, manipulating, and creating related technology for building microbial communities critical to plant health and production (Prasad *et al*., 2018).

This review defines some concepts, shows how microbiota benefits the agroecosystem, and then provides an overview of current microbiome research by highlighting new technologies and approaches.

Microbial niches

Rhizosphere microbiome

The rhizosphere microbiome, which is the narrow zone of soil that is influenced by root secretions (Berendsen *et al*., 2012), includes bacteria, fungi, protists, and oomycetes, is intimately linked to plant's growth, development, and health (Figure 1) (Cai *et al*., 2017; Wu *et al*., 2018). Generally, microorganisms in the rhizosphere can be beneficial or detrimental to the host plant (Yu *et al*., 2019). The harmful microorganisms, such as soil-borne pathogens, diminish plant growth and development, resulting in yield loss and threatening agricultural productivity (Yin *et al*., 2021). Beneficial microbes, on the other hand, can stimulate plant growth and development by enhancing nutrient availability, producing phytohormones, and increasing abiotic and biotic stress tolerance (Rolli *et al*., 2015; Jacoby *et al*., 2017; Yin *et al*., 2021).

Furthermore, the rhizosphere can be divided into two compartments: the ectorhizosphere or rhizoplane; the outer area of soil surrounding the roots and the endorhizosphere, the region in the root which microbes fed by root-derived compounds may colonize, including the root cortex, epidermis, and root hairs (Figure 1) (Kloepper *et al*., 1992; Kumar & Dubey, 2020). In the ectorhizosphere or rhizoplane, microbes that reside on the surface of roots are called epiphytic root microbes. *Actinobacteria, Proteobacteria, Gammaproteobacteria, and Alphaproteobacteria* are known as root epiphytes. In addition, *Burkholderia*, *Pseudomonas*, *Erwinia*, *Sphingomonas* and *Acinetobacter* are the major genera isolated from different plant root surfaces. Few other genera such as *Bacillus*, *Streptomyces, Agrobacterium*, *Nocardia* and *Klebsiella* are also found on some root surfaces. Fungi of class *Ascomycota* represent the eukaryotic root epiphytic community (Butani *et al*., 2021). In the endorhizosphere, endophytic microorganisms, often fungi and bacteria, colonize plant tissue intercellularly and/or intracellularly (Wilson, 1995). Rhizobia, the root-nodule endosymbionts of legume plants, is an endophytic microorganism because of its nitrogen-fixing potential (Gallego-Giraldo *et al*., 2014). *Microbacterium trichothecenolyticumn*, *Brevibacillus choshinensis*, *Endobacter medicaginis*, and *Micromonospora spp* are non-nodulating examples of endophytes (Trujillo *et al*., 2010; Ramírez-Bahena *et al*., 2013; Benidire *et al*., 2017). Endomycorrhizas and ectomycorrhizas are two mycorrhizas that colonize roots as endophytic and ectophytic fungi. The difference between the two types is that ectomycorrhizal fungi's hyphae do not enter individual cells within the root. In contrast, endomycorrhizal fungi's hyphae penetrate the cell wall and invaginate the cell membrane (Allen, 1991). Plant-rhizosphere microbiome interaction is complicated and dynamic (Shi *et al*., 2016). The root exudates, including organic acids, amino acids, polysaccharides, and other primary and secondary metabolites (Bais *et al*., 2006), play a critical role in assembling the rhizosphere microbiome (Backer *et al*., 2018; Zhalnina *et al*., 2018). The release of root exudates is regulated in response to environmental stimuli, and the composition of root exudates varies widely depending on plant species and physiological stage (Phillips *et al*., 2004; De-la-Pena *et al*., 2008; Nguyen, 2009). Root exudates benefit plants by stimulating beneficial microorganisms (e.g., symbionts), improving nutrient uptake, and enabling recognition between self-roots and neighbour-roots (Mommer *et al*., 2016; Meier *et al*., 2017). On the other hand, root exudates can stimulate pathogenic microorganisms; for example, watermelon root exudates increase sporulation and spore germination of *Fusarium oxysporum* f. sp. *niveum* (Hao *et al*., 2010) or microconidia germination of *F. oxysporum* f. sp. *lycopersici* and *F. oxysporum* f. sp. *radicis-lycopersici* increases in the presence of tomato root exudates (Steinkellner *et al*., 2005). These rhizosphere microorganisms can also interact with each other, either competitively or synergistically. For example, non-pathogenic strains of *Streptomyces* can control potato scab caused by *Streptomyces scabies* in a bacterial-bacterial pathogens interaction or *Pseudomonas* spp. can control tomato *Fusarium oxysporum* f. sp. *radicis-lycopersici* in a bacterial-fungal pathogens interaction (Whipps, 2001). An example of antagonist fungal–fungal interaction in the rhizosphere is the interaction between *Phialophora* sp. I-52 and *Gaeumannomyces gramminis* var. *tritici* in wheat (Mathre *et al*., 1998; Whipps, 2001). No clear examples of fungal-bacterial pathogens interaction have been reported (Whipps, 2001).

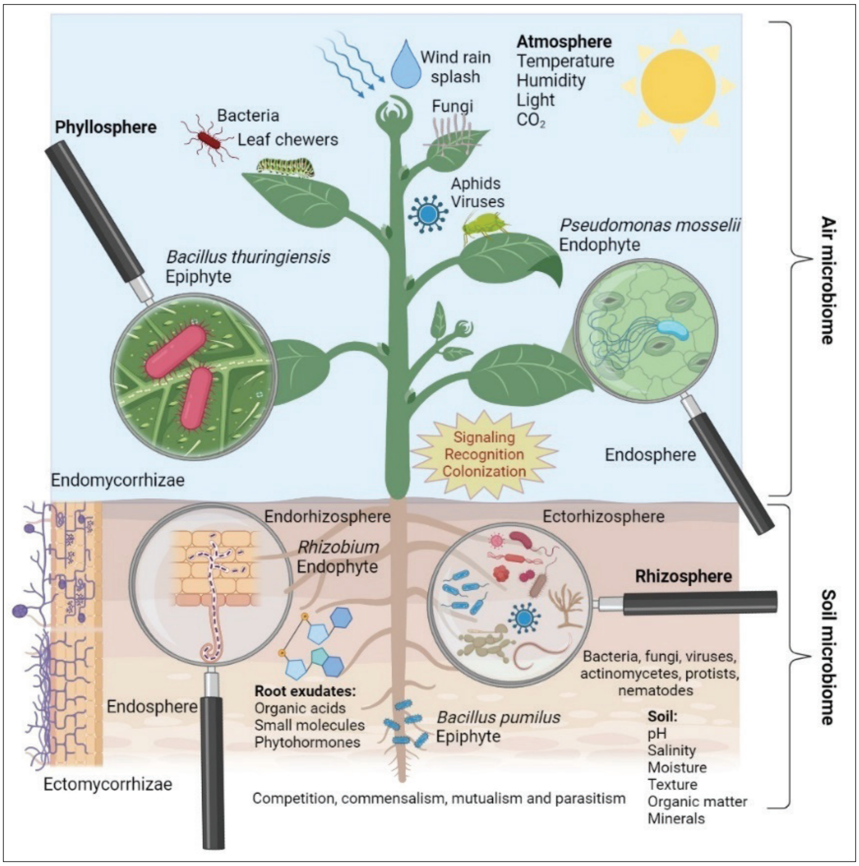

Figure 1: Plant microbiome composition and its association with biotic and abiotic factors

Phyllosphere microbiome

The aerial or aboveground portions of plants, such as stems, leaves, flowers, and fruits, make up the air or aboveground microbiome (Figure 1) (Knief *et al*., 2010). Leaves as the most dominant part form phyllosphere and can be colonized by these microbial communities both on the surfaces (phylloplane; colonized by epiphytic microorganisms) and inside the leaf tissue (endosphere; colonized by endophytic microorganisms) (Vorholt, 2012), resulting in complex interactions between the plant, microorganisms, and the environment (Bashir *et al*., 2022). The other aboveground plant-associated microbial habitats include anthosphere: microbial habitat associated with flowers, carposphere: microbial habitat associated with fruits and caulosphere: microbial habitat associated with stems (Shade *et al*., 2017). The term phyllosphere itself dated to the mid-1950s and was referred to by Jakoba Ruinen in 1961 (Koskella, 2020). Phyllosphere resides in a diversified and dynamic community of bacteria, fungi, viruses, actinobacteria, cyanobacteria, protozoans, and nematodes (Bashir *et al*., 2022). Bacteria outnumber other microorganisms in cell number and taxonomic diversity (Andrews & Harris, 2000).

The establishment of the phyllosphere microbiome depends on the leaf–atmosphere environmental interaction with the residing microorganisms in the phyllosphere (Sivakumar *et al*., 2020). For example, humidity, temperature, nutrient availability (on the plant surface), light/UV intensity, and even soil chemistry determines phyllosphere community composition (Lindow & Brandl, 2003; Leveau, 2015).

Like the rhizosphere, the phyllosphere inhabits both pathogenic and non-pathogenic or even beneficial microorganisms (Montarry *et al*., 2008; Rastogi *et al*., 2013). The phyllosphere microbiome can influence plant growth, development, and health (Balint-Kurti *et al*., 2010; Reed *et al*., 2010; Gerd *et al*., 2011). For instance, some phyllosphere bacteria benefit plants by fixing nitrogen (Leveau, 2015), promoting growth (Bringel & Couée, 2015), serving as a barrier against pathogens, and decomposing the pesticides leftover (Venkatachalam *et al*., 2016). Phyllosphere diazotrophic bacteria utilize carbohydrates excreted by plant leaves (Vorholt, 2012) and fix an appreciable amount of nitrogen which may benefit the plants (Abril *et al*., 2005; Abadi *et al*., 2020). Bentley and Carpenter (1984) discovered that diazotrophic epiphytes provided 10–25% of nitrogen to host plants. For example, some phyllospheric bacteria, indole-3-acetic acid (IAA) producers, such as *Streptomyces fradiae, Bradyrhizobium*, *Azospirillum* and *Rhizobium,* are the bacteria that interfere with the plant hormonal pool by addition of IAA (Spaepen *et al*., 2007). Furthermore, beneficial phyllosphere microorganisms interact with roots and leaves to help plants better tolerate abiotic stress (Ripa *et al*., 2019). For example, inoculation of *Bacillus pumilis* and *Azospirillum lipoferum* increased the abscisic acid concentration in *Arabidopsis thaliana* and maize tissue and stimulated stress-related proteins production (Cohen *et al*., 2015; Yasmin *et al*., 2017). In addition, *Beauveria* fungus on rice leaves can safeguard rice enzyme function while also being an ecologically benign bacterium (Du *et al*., 2014). Generally, the phyllosphere microbiome develops adaptive traits and intimate associations with leaves through mitigating biotic and/or abiotic stress(es) and influencing plant growth and fitness (Vorholt, 2012; Helfrich *et al*., 2018). However, the effects of phyllosphere beneficial or harmful bacteria, or their combinations, on plant performance are poorly known (Traw *et al*., 2007; Saleem, 2015).

Seed microbiome

Microbes can interact with seeds at different plant growth and development stages and form seed microbiomes. The concept of seed microbiome was first developed by Mitter *et al*., (2017). Microbes can inhabit seed interiors (Endosphere) and seed surfaces or zone of soil under the direct influence of germinating seeds, the so-called spermosphere. Seed microbiota can be in the seed coat, endosperm, and embryo (Figure 2). The seed microbiome makeup can considerably influence seed quality (Links *et al*., 2014). For example, the negative effect of pathogenic microbes on seed germination is the most common (Munkvold, 2009). For example, Rybakova *et al*., (2017) discovered a link between pathogen susceptibility and seed microbiome diversity and abundance. By contrast, some seed-borne microbes, on the other hand, can improve germination rates by releasing seed dormancy via cytokinin synthesis (Goggin *et al*., 2015) or promote plant growth by releasing IAA and 1-aminocyclopropane-1-carboxylate (Chimwamurombe *et al*., 2016). It has been demonstrated that artificially inserting plant growth-promoting microbes into wheat seeds may improve crop yield (Mitter *et al*., 2017).

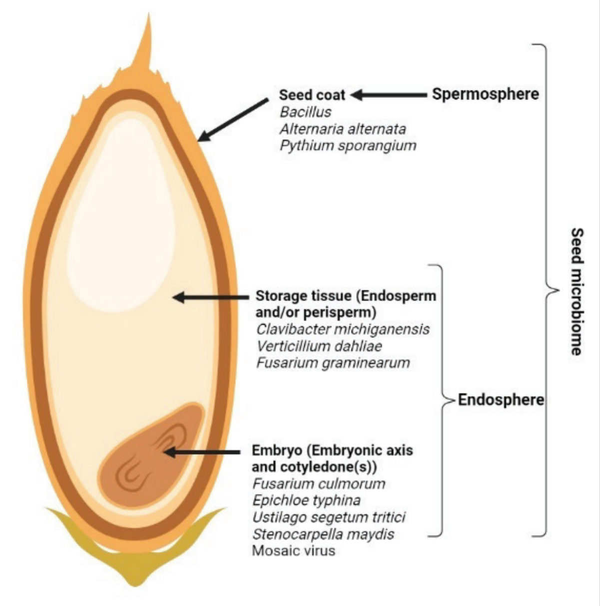

Figure 2: Seed microbiome and localization of some seed-associated microorganisms

Seed microbiomes have a varied makeup, with up to 9000 microbial species (Shade *et al*., 2017). A variety of endophytic bacteria have been discovered in seed microbiomes. Bacteria colonizing inside seed tissue are mainly from the phyla *Actinobacteria*, *Proteobacteria*, *Firmicutes*, and Bacteroidetes. However, the most common genera are *Enterobacter*, *Pantoea*, *Pseudomonas*, *Sphingomonas*, and *Acinetobacter* (Hardoim, 2019; Johnston-Monje *et al*., 2021). Fungi that inhabit the seeds can be categorized as clavicipitaceous endophytes and non-clavicipitaceous endophytes. The former are endosymbionts transmitted by grass seeds and include *Atkinsonella*, *Balansiopsis*, *Balansia*, *Dussiella*, *Myriogenospora*, *Epichloë*, *Parepichloë*. The latter, which are mainly Ascomycetes and Basidiomycetes, can be seen in a variety of seeds and includes *Alternaria*, *Cladosporium*, *Fusarium*, *Aspergillus*, *Undifilum*, *Rhizoctonia*, *Chaetomium*, *Colletotrichum*, *Phialophora*, *Epicoccum*, *Tricothecium*, *Cryptococcus*, and *Filobasidium* (Hardoim, 2019; Johnston-Monje *et al*., 2021).

Seed microbiome composition is governed by plant species or genotype, environmental conditions, and management procedures (Berg & Raaijmakers, 2018). In a study by Moreira *et al*., (2021), the seed microbiome of wheat, canola, and lentil lines across two generations were profiled, and crop, genotype, and the environment were reported as the key drivers of the seed microbiome assemblage.

Most seed microbiome research has focused on culturable microbiota, endophytes, and microbial community comparisons across cultivars, geographical regions, and agricultural management methods, whereas seed microbiome persistence across crops and generations, is largely unknown (Khalaf & Raizada 2016; Sánchez-López *et al*., 2018). Similarly, bacteria have gotten more attention than fungi, and few studies look at both microbiomes (Barret *et al*., 2015). As a result, our understanding of the seed microbiome is still in its early stages, and its potential for improving plant health and production is mainly unexplored.

Benefits of microbiomes for agroecosystem

Microbiome and plant growth and development

Plant microbiomes form a complex and interconnected microbial network (Huang *et al*., 2020). Each organism performs a unique role critical to plant health and ecosystem function (Zhou *et al*., 2010). For instance, Shi *et al*., (2016) described that increased complexity and connectivity of rhizosphere network are qualities of the rhizosphere bacterial communities.

Plant growth, development and health can be influenced directly by microorganisms that produce phytohormones, improve nutrient uptake and indirectly by triggering the plant immune responses or outcompeting and suppressing plant pathogens, for instance, through the synthesis of fungal cell wall–degrading enzymes, antibiotics, and siderophores (Gamalero & Glick, 2011; Berendsen *et al*., 2012; Gu *et al*., 2020).

Beneficial microorganisms, mainly bacteria named plant growth-promoting microbes (PGPMs), can stimulate plants' growth by altering plants' hormonal and metabolic balance. The secretion of secondary microbial metabolites, which act as hormone-like plant growth regulators, and the synthesis of proteins involved in plant defense signaling enable microbes to colonize plant tissue (Manganiello *et al*., 2018; Stringlis *et al*., 2018). Many bacteria and fungi associated with plants can produce IAA, directly impacting plant growth and development (Garnica-Vergara *et al*., 2016). PGPMs that produce auxins have provoked transcriptional changes in the defence-related hormone. Genes involved in cell wall biosynthesis (Spaepen *et al*., 2014) cause longer roots (Hong *et al*., 1991), increase root biomass and reduce stomata size and density (Llorente *et al*., 2016), and turn on auxin related genes that boost plant growth (Ruzzi & Aroca, 2015). In addition to auxin-mimicking compounds, gibberellins, cytokinins, and defense-related hormones, such as jasmonic acid-isoleucine or salicylic acid, are primarily produced to modulate plants immunity and facilitate microbial colonization (Stringlis *et al*., 2018). Cytokinins can enhance root exudate production and potentially increase plant-microbe signaling (Ruzzi & Aroca, 2015; Backer *et al*., 2018). Gibberellin-producing PGPMs species such as *Bacillus cereus*, *Promicromonospora* sp. *Leifsonia soli* and *Enterococcus faecium* have been shown to stimulate shoot growth in plants deficient in gibberellin synthesis (Joo *et al*., 2005; Kang *et al*., 2014). This indicates that these species compensate for the absence of plant gibberellins with added bacterial gibberellins (Lee *et al*., 2015). It was stated that many beneficial microorganisms promote plant growth by lowering plant ethylene levels through the bacterial enzyme 1-aminocyclopropane-1-carboxylate (ACC) deaminase, which hydrolyses the ACC (immediate ethylene precursor) into α-ketobutyrate and ammonia (Glick, 2014). Several studies have demonstrated that inoculating plants with PGPMs, which produce ACC deaminase, improves stress tolerance. This appears to be the case because PGPMs can prevent ethylene levels from rising to suppress plant growth (Ahemad & Kibret, 2014; Pérez-Montaño *et al*., 2014; Ruzzi & Aroca, 2015).

Although numerous studies have confirmed the role of microorganisms and a healthy microbiome in sustainable agriculture and the environment, plants' intracellular signalling in response to various phytohormones generated by PGPMs and their interactions with endogenous plant pathways are still poorly understood. This knowledge appears to be required to anticipate the effects of PGPMs application, mainly when we are talking about the microbiome, where several microorganisms are involved and release several phytohormones. Therefore, further research is needed to discover whether biotechnological techniques can provide predictable results.

Microbiome and soil fertility and nutrients use efficiency

As an important component of the soil ecosystem, the soil microbiome plays a essential role in maintaining soil fertility and is used as an ecological indicator to assess soil health. Unfortunately, modern and intensive agriculture drastically affects soil microbial abundance, diversity and activity. Excess chemical application, fertilizers or pesticides and a low amount of soil organic matter are the main threat to the microbiomes. Numerous studies have examined the effects of chemical nitrogenous fertilizers on soil microbiome and found that chemical fertilizers appear to suppress microbial community (Treseder, 2008; Zhou *et al*., 2017; Xie *et al*., 2019). For example, Reid *et al*., (2021) found that rhizobacteria abundance and populations were decreased in wheat grown in soils treated with NPK fertilizer.

Applying beneficial microbes in agroecosystems as a sustainable method for long-term nutrient availability could mitigate the excess use of chemical inputs (Antar *et al*., 2021). Soil microorganisms, mainly bacteria and fungi, have long been known to contribute to plant nutrition. Nitrogen is an essential mineral nutrient for most plant physiological functions, including photosynthesis, amino acids, and protein biosynthesis (Alori *et al*., 2017). Some members of the plant microbiome can significantly minimize the requirement for soil nitrogen supplementation, either directly through legume and rhizobium symbiosis or indirectly by supporting nitrogen fixers by their secretions (Naamala & Smith, 2020). Nitrogen fixers microorganisms are classified into two major groups based on their type of association formed with plants: the symbionts and the free-living nitrogen fixers. The symbiotic nitrogen fixers consist of *Rhizobium*, *Sinorhizobium*, *Azoarcus*, *Mesorhizobium*, *Allorhizobium*, *Frankia*, *Bradyrhizobium*, *Azorhizobium*, *Burkholderia*, and some *Achromobacter* strains (Babalola, 2010; Pérez-Montaño *et al*., 2014; Turan *et al*., 2016). The free-living nitrogen fixers consisted of *Azoarcus*, *Herbaspirillum*, *Azospirillum*, *Gluconacetobacter*, and *Azotobacter* (Vessey, 2003). In symbionts, the formation of root nodules, specialized structures where atmospheric nitrogen is reduced into useable forms (primarily NH_3), starts with crosstalk between rhizobia and legume in the form of signal compounds (Naamala *et al*., 2016) while free-living nitrogen-fixing bacteria thrive in the phyllosphere and rhizosphere of plants (Hardy & Holsten 1973). Foliar and root application of PGPMs has increased nitrogen content (Luziatelli *et al*., 2019). For example, foliar application of *Azotobacter chroococcum* and *Azospirillum lipoferum* as biofertilizers resulted in considerable increases in aerial plant height, leaf number and fresh weight in lettuce (Hanafy Ahmed *et al*., 2002). It has also been documented that the presence of *Azotobacter* species in the soil improves nitrogen availability (Din *et al*., 2019) and phosphorous (Velmourougane *et al*., 2019).

The soil microbiome has vast potential for increasing soil phosphorus availability and is an important regulator of phosphorus transformation processes (McRose *et al*., 2021). For example, organic acids like gluconic acid and 2-ketogluconic acid are produced by rhizosphere bacteria such as *Pseudomonas*, *Rhizobium*, *Bacillus*, and *Enterobacter* solubilize phosphates (Werra *et al*., 2009; Adeleke *et al*., 2017). Moreover, arbuscular mycorrhizal symbiosis, an essential prerequisite for phosphorus acquisition from the soil, contributes to plant growth and health (Wen *et al*., 2019).

Soil microorganisms have also been identified to contribute to plant iron nutrition by producing organic compounds called siderophores (Mohanty *et al*., 2021). Several bacterial species can produce siderophores, including, *Azospirillum*, *Klebsiella*, *Pantoea*, *Pseudomonas*, *Azotobacter*, *Bacillus*, and *Serratia* (Leontidou *et al*., 2020). In a study, the iron content of sunflower, maize (Masalha *et al*., 2000), rape, and red clover (Jin *et al*., 2006) was considerably lower when grown in sterile rather than sterile non-sterile soil. As a result, PGPMs offer a cost-effective and safe alternative technique for increasing soil fertility and enhancing plant growth and development.

Microbiome and abiotic stresses

As the effects of climate change become more apparent over time, severe environmental conditions such as drought, salinity, low and high temperatures, and nutrient deficiency are becoming more frequent. Among these environmental conditions, drought and heat stress cause notable losses in major cereal species such as wheat, barely and maize (Vogel *et al*., 2019). Under such stressful conditions, plants' metabolism is disturbed by several biochemical factors. To resist such circumstances, metabolic reconfiguration is essential to satisfy the need for anti-stress factors such as antioxidants, solutes, and proteins (Obata & Fernie, 2012). Hence, employing a long-term strategy to increase plant tolerance to environmental constraints is critical for ensuring and optimizing global food production. The use of PGPMs in a sustainable manner is one of the eco-friendly approaches that can support plants to resist severe environmental conditions (Shah *et al*., 2021). Most PGPMs can produce phytohormones that stimulate plant growth and crop yield under different abiotic stress (Sarkar *et al*., 2018; Jochum *et al*., 2019; Mellidou *et al*., 2021). In addition, the plant microbiota involved in the production of phytohormones stimulates the production of osmolytes, antioxidants, regulation of stress-responsive genes, and alteration of root morphology, which results in drought tolerance in plants. Therefore, the PGPMs conserve plant growth under drought stress, leading to increased agricultural productivity (Subiramani *et al*., 2020).

Heat stress impacts plants' various characteristics, including germination, growth, and the production of flowers and seeds through biochemical, physiological, molecular, and morphological changes (Siddiqui *et al*., 2015). Biochemical changes include changes in amino acids, metabolites, osmolytes, hormones, reactive oxygen species generation and antioxidant enzymes. Molecular alterations include the changes in genes involved in the expression of Osmo protectants, detoxifying enzymes, transporters, and upregulating the expression of heat shock proteins and stress-induced proteins (Khan *et al*., 2020).

Heat stress tolerance can be enhanced by breeding, tissue culture technologies and nutrients management; however, these approaches are time-consuming, expensive, and have negative environmental consequences (Jan *et al*., 2018). PGPMs are an alternative and environmentally friendly solution to improve crop productivity by reducing the detrimental effects of heat stress (Park *et al*., 2017). Several studies have previously described the use of PGPMs to improve heat stress tolerance in plants such as sorghum (Ali *et al*., 2009), chickpea (Srivastava *et al*., 2008), wheat (Ali *et al*., 2011; Abd *et al*., 2014) and tomato (Issa *et al*., 2018). Recently Khan *et al*., (2020) identified thermotolerant *Bacillus cereus* SA1–a bacterium that can produce biologically active metabolites such as gibberellins, indole-3-acetic acid and organic acids – could improve biomass, chlorophyll fluorescence and chlorophyll content in soybean grown under normal and heat stress treatments. PGPMs could be a practical approach to overcoming the salinity problem. Bacteria species such as *Azotobacter*, *Azospirillum*, *Bradyrhizobium*, *Rhizobium*, *Bacillus*, and *Pseudomonas*, isolated from the saline soils, could be used in salt-stressed farmlands. These salinity resistant PGPMs attach osmotolerance to the plants and provide several benefits such as improved growth, better nutrient uptake, increased chlorophyll content, enhanced vigour and yield (Subiramani *et al*., 2020). Under salinity stress, *Enterobacter* sp. could help rice seedlings cope with salinity stress (Sarkar *et al*., 2018). Similarly, salt-tolerant *Pseudomonas fluorescens* increased plant growth in maize (Zerrouk *et al*., 2016). In wheat, inoculation with *Bacillus aquimaris* improved salt tolerance and plant growth (Upadhyay *et al*., 2011). Also, *Bacillus subtilis* mitigated salinity stress in white clover (Han *et al*., 2014).

Microbiome and biotic stresses

Bio-stressors are one of the many challenges that modern agriculture encounters. Plant pathogens such as viruses, bacteria, and fungi are among the primary biotic stressors severely reducing crop production. There are various instances when the plant microbiome, particularly the root and endophytic parts, plays a vital role in suppressing diseases of crops (Mendes *et al*., 2011; Spence *et al*., 2014; Cha *et al*., 2016). For example, Yang *et al*., (2020) revealed bacterial blight-induced complexed changes in rice leaves' endophytic fungal and bacterial communities. They demonstrated that bacterial blight-associated enrichment of some endophytic bacterial taxa, e.g., *Pantoea* sp. isolates, may play critical roles in controlling the development of bacterial blight disease in rice. Control of *Rhizoctonia solani* of sugar beet was because of *Pseudomonas* spp. (Mendes *et al*., 2011).

Similarly, Koberl *et al*., (2013) used a combination of 45 *Bacillus* spp. with *Streptomyces* in managing *Ralstonia* disease in medicinal plants. Inoculation with *Trichoderma harzianum* has been shown to have an antagonistic impact on fungal diseases (Monteiro *et al*., 2010). Key members of the wheat microbiome can improve fusarium head blight (*Fusarium graminearum*) resistance by interacting with wheat traits to shape the overall wheat phenotype and cultivar performance (Karlsson *et al*., 2021).

The rhizosphere microbiome can control nematodes by phytohormone synthesis, nitrogen fixation, phosphorus and potassium solubilization, siderophores containing iron, and creating a gaseous form of nitrogen like ammonia (Mahmud *et al*., 2021). Microorganisms can also be used in controlling insects. For example, *Metarhizium* is a soil-inhabiting insect-pathogenic fungus worldwide and is currently used as a biocontrol agent against crop pests (Sasan & Bidochka, 2012). Other fungi known as insect pathogens include *Acremonium*, *Beauveria*, *Cladosporium*, *Clonostachys*, and *Isaria* (Wang *et al*., 2005).

A healthy microbiome can control plants disease through different mechanisms such as accumulating the stress-related phytoalexin metabolites, regulating the defense-related gene expression, inducing the oxidative burst (Gruau *et al*., 2015), by non-producing lipopeptides and overproducing surfactin (Farace *et al*., 2015), inducing defences modulated by Ca^{2+} signalling (Vatsa-Portugal *et al*., 2017) and producing ACC deaminase enzyme that helps plants regulate the stress-related hormone ethylene level (Gamalero *et al*., 2017).

However, the Phyllosphere microbiome is often overlooked and plays an essential role in plant health (Gao *et al*., 2021). Recent investigations have found that as the rhizosphere microbiome plays a crucial role in determining aboveground health (Fitzpatrick *et al*., 2018), infections with aboveground pathogens change the microbial community in the plant's rhizosphere (Berendsen *et al*., 2018; Yuan *et al*., 2018) suggesting that under- and aboveground microbiomes are systematically linked (Gao *et al*., 2021). However, no comprehensive study of the potential connection between under- and aboveground microbiomes has been performed.

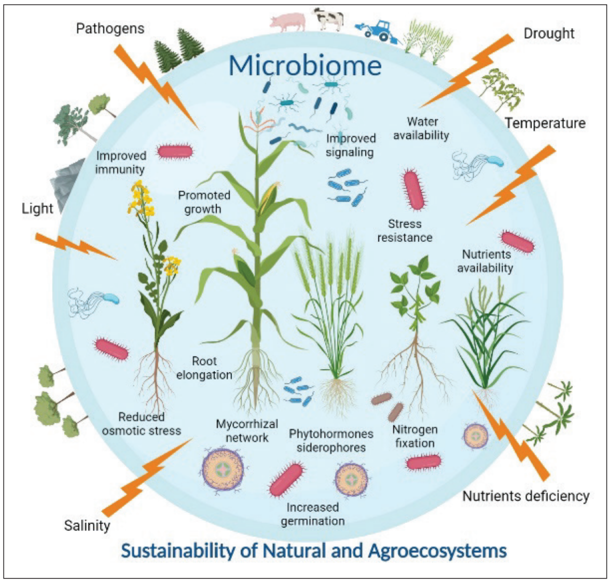

Figure 3: Microbiome for crop improvement and sustainability

Overall, the plant microbiome acts like a second immune system, protecting the plant against biotic stresses, can promote plant growth, assisting the plants acquire nutrients, or protecting against abiotic stress such as drought, heat, or salinity (Figure 3), finally leads to improved plant health and productivity. Therefore, significant attention must be paid to microbiome studies to help develop and implement beneficial microbes in plant protection and sustainability promotion.

Microbiome research on major crops

Microbiome studies have always been limited by the small number of experiments, variations in sampling and analysis techniques, and small sample sizes within each experiment, but advances in new technologies, for example, high-throughput DNA sequencing coupled with omics analyses, have enabled a revolution in plant microbiome studies and accelerated this area of research. The most general microbial genomics approaches include 16S/18S/ITS amplicon sequencing, metagenomics, meta-transcriptomics, microbial whole-genome sequencing, complete plasmid sequencing, and microbial single-cell sequencing. Using high-throughput and high-resolution genomics technology platforms, microbial genomics techniques can enhance agricultural productivity through soil microbial diversity study, functional microbial genomics, biological product creation, and bacterial genome editing. Next-generation sequencing (such as Illumina HiSeq/MiSeq and Roche 454) and PacBio SMRT sequencing are suitable options for microbial research. PacBio SMRT systems may analyse methylation with PacBio analytical tools and provide long-length readings.

Most of the recent microbiome work involves work on plants in natural and agroecosystems using a combination of field- and lab-based studies, molecular/computational techniques, and functional assay to better understand the plant microbiome, including their community assembly, function transmission and dispersal. DNA sequencing has been applied to overcome challenges in identifying and monitoring plant microbiome (Nezhad, 2014). This method mainly relies on amplifying and sequencing small, conserved regions from microbes' genomes, including 16S ribosomal RNA sequences for bacteria and the internal transcribed spacer region or *Elongation Factor 1 alpha* (*EF1α*) and *Beta-tubulin* genes for fungal species (Raja *et al*., 2017). In this context, Hu *et al*., (2019) applied the whole genome shotgun sequencing as a method for the detection of wheat fungal pathogens. They diagnosed fungal pathogens, including *Puccinia striiformis* f. sp. *Tritici* (causal agent of wheat stripe rust), *Zymoseptoria tritici* (causal agent of septoria tritici blotch), and *Pyrenophora tritici repentis* (causal agent of yellow leaf spot). They also identified the bacterial genus *Pseudomonas* co-present with *Puccinia* and *Zymoseptoria* but not *Pyrenophora* infections.

Recently, culture-independent and culture-dependent metagenomics approaches have been employed to investigate the whole rhizosphere microbiome of many crops such as wheat (Ofek-Lalzar *et al*., 2014), barley (Bulgarelli *et al*., 2015) and rice (Zhang *et al*., 2019). According to culture-independent methods, *Proteobacteria* account for over 40% of the bacterial population in the wheat rhizosphere. *Acidobacteria*, *Actinobacteria*, and *Bacteroidetes* are the other dominant phyla (10–15%), with the other phyla accounting for < 5% each (Tkacz *et al*., 2020; Prudence *et al*., 2021), whilst culture-dependent techniques revelated that *Actinobacteria* and *Proteobacteria* (25% each) and then *Firmicutes* (10%) are the main phyla in the wheat rhizosphere (Germida & Siciliano, 2001). Furthermore, Comby *et al*., (2016) reported that Ascomycota (mainly Sordariomycetes or Dothideomycetes) is the most dominant fungal phylum, followed by Basidiomycota (with dominance by Agaricomycetes in which Polyporales and Russulales were top orders), and Zygomycota. In a similar study, MinION oxford nanopore sequencing was used, and the prevalent phyla were recognized as *Proteobacteria* (68%), *firmicutes* (13%), *bacteroidetes* (3%), *actinobacteria* (3%) and *acidobacteria* (3%) (Srivastava *et al*., 2020).

In a study, Wang *et al*., (2021) used metagenomic sequencing and analysis and showed a significant microbiome difference between wheat rhizosphere and bulk soil. Similarly, using pyrosequencing of bacterial 16S rRNA genes, Peiffer *et al*., (2013) reported a substantial variation in bacterial richness, diversity, and relative abundances of taxa between bulk soil in the maize rhizosphere. Beirinckx *et al*., (2020) identified 12 abundant and enriched bacterial families that colonize maize roots using the same method. The microbiome structure in the bulk soil and soybean rhizosphere as affected by crop sequences was investigated by Bolaji *et al*., (2021) using a combination of marker-based Illumina sequencing and bioinformatics analyses. They demonstrated that bacterial species richness and evenness in the soybean rhizosphere soil were similar following canola and soybean compared to a previous corn sequence. On the other hand, fungi richness and evenness were unaffected by crop sequence. Floc'h *et al*., (2020) used a long-term field experiment. They sampled the rhizosphere of canola grown in different rotation sequences and detected only one core bacterial amplicon sequence variant in the prokaryotic component of the microbiota of canola rhizosphere, a hub taxon identified as cf. *Pseudarthrobacter* sp. Lay *et al*., (2018) extracted DNA from canola roots and nearby rhizosphere soil and constructed MiSeq amplicon libraries, focusing on bacterial and archaeal 16S rRNA genes and the fungal ITS regions. They found that the root and rhizosphere microbiome of canola differed from wheat and pea. These microbiomes include several putative PGPM, including *Amycolatopsis* sp., *Serratia proteamaculans*, *Pedobacter* sp., *Arthrobacter* sp., *Stenotrophomonas* sp., *Fusarium merismoides*, and *Fusicolla* sp., which correlated positively with canola seed yield.

Unmapped DNA sequencing reads as microbial reads were used to characterize the structure of the rice bacterial microbiome by Roman-Reyna *et al*., (2020), who identified central bacteria taxa that appear as microbial hubs and may impact the network of host-microbe interactions. They found that the genera *Xanthomonas, Pseudomonas*, *Burkholderia* and *Mycoplasma* were the most abundant bacteria in the rice leaf microbiome. They also discovered regions in the rice genome that might be involved in forming these microbial hubs. Using 16S rRNA gene-based metagenomic analysis and Illumina MiSeq sequencing, Oliveira *et al*., (2022) investigated rice filed rhizosphere bacterial diversity and composition. They reported 56 Phyla, with four major Phyla: *Acidobacteria*, *Proteobacteria*, *Bacteroidetes* and *Actinobacteria*.

Therefore, we can use these methods to understand the relationships between microbial communities and plants and increase farm productivity in an environmentally sustainable approach.

CONCLUSION AND OUTLOOK

The plant microbiome and plant-microbe interaction studies can lead to potential farming methods that are both environmentally friendly and agronomically sustainable. Furthermore, microbiome study plays a crucial role in developing biofertilizers, biocontrol and bioremediation agents in sustainable agriculture. Despite the abundance of research on plant and soil microbiome and plant-microbe interactions, there is not much on the molecular mechanisms that underpin gene activity and signal transduction during beneficial and harmful interactions. Hence, understanding the genetics of microbiota and its interaction with crops using novel technologies such as next-generation sequencing and omics technologies is essential for providing broad and in-depth knowledge of the biological phenomenon to improve plant health, disease control and food quality (Kankanala *et al*., 2019). Due to genomics tools, our understanding of the plant microbiome has dramatically increased in the post-genomic era. Nonetheless, integrated approaches, such as multi-omics and microbiome engineering methodologies, are still necessary to substantially improve our understanding of the structure and dynamics of microbiomes. Multi-omics data integration could assist in discovering the molecular mechanisms underlying microbiome recruitment, also considering metabolite–metabolite, microbe-microbe, and metabolite–microbe interactions. In addition, integrating efficient approaches with emerging precision agriculture, artificial intelligence, synthetic biology, satellite, and big data can offer a robust framework to achieve the actual potential of plant microbiome in agriculture and environmental science. Nevertheless, numerous obstacles in this field of study must be overcome to understand the microbiome comprehensively. These hurdles are mainly identifying the molecules and compounds responsible for the network communication between plants and microorganisms, taking bacterial and fungal communities to the next level, expanding and validating microbial cultures, understanding plant genotype, microbiome, and environment interactions, taking advantage of host-mediated microbiome member and function selection and developing novel methods and techniques for practical application and manipulation in agricultural settings.

Moreover, there is substantial evidence that plant genetics affects the microbiome. Therefore, plant breeding programs should take the importance of the microbiome as a lifelong strategy. These suggested strategies will offer alternatives for investigating and leveraging plant microbiome to improve global agriculture's sustainability and productivity. The authors believe the microbiome's full use will be realized when major fertilizer companies worldwide take up R&D in this field and produce novel microbial fertilizers that would be the next green revolution. Indeed that should be called the 'true green revolution' where inputs are environmentally friendly, clean and harmless to the fauna and flora, including our water table. The authors envision the leap from research to products as an agricultural transformation that all world habitants benefit from.

Conflict of interest

The authors declare no conflict of interest in this article.

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RESEARCH ARTICLE

Artificial intelligence framework for threat assessment and containment for covid-19 and future epidemics while mitigating the socioeconomic impact to women, children, and underprivileged groups

G Ilangarathna[1], H Weligampola[1], Y Ranasinghe[1], E Attygalla[2], R Godaliyaddha[1], V Herath[1], P Ekanayake[1], SY Ekanayake[3], M Pinnawela[3], S Dharmaratne[4], G Tilakaratne[5] and J Ekanayake[1*]

[1] *Faculty of Engineering, University of Peradeniya, Peradeniya, Sri Lanka.*
[2] *Faculty of Engineering and Physical Sciences, University of Surrey, Guildford, Surrey, United Kingdom, GU2 7XH.*
[3] *Faculty of Arts, University of Peradeniya, Peradeniya, Sri Lanka.*
[4] *Faculty of Medicine, University of Peradeniya, Peradeniya, Sri Lanka.*
[5] *Institute of Policy Studies, Colombo, Sri Lanka.*

Abstract: With the emergency situation that arises with COVID-19, the intense containment strategies adopted by many countries had little or no consideration towards socio-economic ramifications or the impact on women, children, socio-economically underprivileged groups. The existence of many adverse impacts raises questions on the approaches taken and demands proper analysis, scrutiny and review of the policies. Therefore, a framework was developed using the artificial intelligence (AI) techniques to detect, model, and predict the behaviour of the COVID-19 pandemic containment strategies, understanding the socio-economic impact of these strategies on identified diverse vulnerable groups, and the development of AI-based solutions, to predict and manage a future spread of COVID or similar infectious disease outbreaks while mitigating the social and economic toil. Based on generated behaviour and movements, AI tools were developed to conduct contact tracing and socio-economic impact mitigation actions in a more informed, socially conscious and responsible manner in the case of the next wave of COVID-19 infections or a different future infectious disease.

Keywords: Artificial intelligence, COVID-19, socio-economic impacts.

INTRODUCTION

The novel coronavirus named Severe Acute Respiratory Syndrome-Coronavirus-2 (SARS-CoV-2), was first recognized in an outbreak in Wuhan, China in December 2019 and has since then swept the modern world into unprecedented turmoil. The World Health Organization (WHO) was compelled to declare the coronavirus disease-2019 (COVID-19) a pandemic on March 11, 2020, when the total number of cases amounted to more than 118,000 with the disease spread in over 110 countries worldwide.

COVID-19 essentially pushed human civilization to a standstill. This global crisis tested the human response as communities, localities, countries, regions and the entire world (WHO, 2020). Almost all the affected countries initiated social distancing protocols with movement restrictions and eventually went into some form of a "lock-down". However, different countries adopted different measures to contain the spread of the virus. In Sri Lanka, the first patient with COVID-19 was reported on January 27, 2020, a Chinese female visiting the country. When the first local patient was reported on March 11, Sri Lanka initiated rigorous measures to reduce the spread of the disease. The government suspended all arriving international flights and ships while imposing a nationwide curfew on March 20. In selected districts and some designated areas in some districts, curfew was continued till June 6, whereas in other districts curfew was relaxed periodically. Curfew

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[*] Corresponding author (jbe@ee.pdn.ac.lk; https://orcid.org/0000-0003-0362-3767)

was relaxed systematically and from June 28th, curfew was lifted countrywide.

There are numerous claims where individuals and groups were subjected to excessive pressure, stress, discomfort or discrimination due to some of the measures adopted for the sake of combatting the propagation of COVID-19 (ILO, 2020; FAO, 2020; UN WTO, 2020). In other words, the impact of the COVID-19 pandemic, as well as the adopted containment strategies, had a disproportionate and inhomogeneous impact over various demographic and socio-economic groups of classification in the society. Such notable identifiable groups include women (who hold around 70% of the jobs in the health and social care sectors and around 54% in the tourism sector), informal service sector workers, labour-intensive manufacturing and construction sector workers, informal economy workers, casual and temporary workers, informal workers in the agriculture and food supply sector, younger and elderly workers, refugees and migrant workers, micro-entrepreneurs, and the self-employed.

Especially, the impact of containment measures taken by policymakers was severe on women, children, and the above-mentioned socio-economically underprivileged groups (called 'identified diverse groups' hereafter) (UN WTO, 2020; UNICEF, 2020). The mere existence of such issues raises questions on the approaches taken and demands proper analysis, scrutiny, and review of the policies. This would be beneficial in formulating the response towards addressing not only the future progress of COVID-19 but also any other future similar crisis of global scale.

Typically, even in the data-driven systematic analysis of a problem, the concerns of women, children, and socio-economically underprivileged are under-represented and/or simply lost in the process of averaging out. Therefore, the special and unique concerns of these groups remain invisible or unrecognized. Through a 3000-household survey (*https://covid.eng.pdn.ac.lk/household.php*) conducted to understand the broader impacts of COVID-19 outbreak and to identify those segments of the population that are disproportionately affected by the pandemic, it was found that the overall policy frameworks fail to address the issues and concerns of 'identified diverse groups.'

Considering the impact of emergency containment measures (such as open, social-distancing, restricted, lock-down, and shut-down) taken by the governments worldwide to combat COVID 19 on a diverse group of people based on occupation (such as daily wage labourers, mass factory workers, domestic workers, ad-hoc workers, and self-employed personal) and occupancy (such as urban slums, urban multi-storey flats, and metropolitan setups), it is vital to investigate the socio-economic impact on the 'identified diverse groups.' Having understood such impacts, this paper discusses, the development and use of artificial intelligence (AI) techniques to detect, model, and predict the behaviour of 'identified diverse groups' under COVID-19 pandemic containment strategies; understanding the impact of these strategies initiated in Sri Lanka; and the development of AI-based solutions, to predict and manage a future spread of COVID or similar infectious disease outbreaks. Socio-economic impact due to COVID-19 has been studied with respect to several isolated factors such as economic activities, health and poverty. For example, a study has been conducted in Nigeria (Obi *et al*., 2020) comprising of 300 respondents to collect information on the impact on economic activities only whereas, in our proposed work, a larger population of respondents is involved in the survey. In another case study conducted in San Francisco Bay area (Martin *et al*., 2020), the impact on households and poverty has been analysed under different containment policies through mathematical modelling using past data. However, this does not reflect the real scenario during the pandemic as there was an abrupt change in employment and economic activities. Consequently, we address the gap of current information by collecting data on employment covering periods before and during the pandemic. In addition, the impact on health programs in India has been analysed in (Gopalan & Misra, 2020). This has been done using historical census data and COVID-19 reports rather than reaching out to the public to collect information on their concerns. In the proposed work, we dedicate a section to collecting data on selected health programs to analyse the impact of COVID-19 on essential sections of the health sector. Moreover, these studies use statistical analysis (Ozili, 2020; Das *et al*., 2022) to make interpretations and develop passive measures instead of using them to provide proactive measures. In the proposed research, the data collection will facilitate an AI to model, forecast, and emulate different strategies to lessen the adverse effects of COVID-19 on socioeconomics.

METHODOLOGY

This project has a number of stages namely, data collection, data analysis, simulator design, analysing the disease spread using the simulator, and generating outputs such as impact of different containment measures. Initially, the data such as census data, visual data (satellite image data), and infectious data from publicly available databases for

Sri Lanka and many other countries as available were collected. Then, according to the current status, a deep learning-based forecasting tool was developed to predict the number of cases and deaths fourteen days ahead which is robust under fluctuating testing conditions and limited infection data. An impact analysis was conducted to better understand the interplay between a region's demographics and its possible susceptibility to being impacted by a pandemic. In addition, a correlation study has been carried out using background data and case data of different nationalities to find a relationship between demography and cases. Further, the satellite images were used to find a correlation between aerial visuals and socio-economic data to map to covid threats as this would facilitate an alternate means for region-based containment strategies to be devised based on visual information.

An island-wide survey of 3000 households (about 12000 individuals) was conducted representing a diverse population based on gender, ethnicity, economy, etc. The household survey was carried out by using Face-to-face CAPI (Computer Aided Personal Interviews) (Lavrakas, 2008) using a structured questionnaire (Scott & Usher, 2004; Lavrakas, 2008). The questionnaire was designed to cover questions related to demographic characteristics of the household (age, gender, occupation, etc of each household member), geographical location, broader effects of COVID-19 on household (whether or not any household member experienced any effect on health, livelihoods, income, etc.). The questionnaire was translated into local languages, ethical clearance was obtained from the ethical committee of the Faculty of Arts of the University of Peradeniya and was piloted (Sinhala/ Tamil) before conducting face-to-face Computer-Assisted Personal Interviewing (CAPI). The second phase of the task, which is ongoing, will commence holding focus group discussions. For this, approximately 216 individuals are invited to participate from the people who partook in the island-wide survey. The discussions are conducted to collect more detailed information on the socio-economic impact due to the pandemic, which was not covered in the national survey. Further, roughly 242 individuals are selected from the survey data to represent various employment/occupational categories. The interviews are used to collect data on mobility and behaviour patterns of the participants before, during and after the pandemic. The interviews happen in parallel with the focus group discussions. In the meantime, 150 interviewees are involved to collect data on present movement patterns using a positioning app installed on mobile phones.

As future work, all data collected will be organized to use with artificial intelligence techniques. Before applying the AI techniques, the focus will be placed on reconstructing missing and partial data, enhancing the resolution of data and pre-processing of data if/when required from the data collection tasks. For example, smoothing and reconstruction techniques will be applied to estimate missing time series data for case data to mitigate the impact of fluctuating or non-uniform testing patterns. Then, hyperspectral satellite data will be used to generate high-resolution information maps to better link it to the collected socio-demographic information and heatmaps will be generated from case data to better display covid severity visually.

After data collection and organization, AI tools will be used for collision estimation, clustering, threat assessment, and generalized representations. Specifically, the emulation engine that is being developed will assess the disease propagation from and to different occupational sectors, locations, and social groups based on movement, behaviour and interaction modelling at the population level. Furthermore, case forecasting or pandemic threat assessment algorithms will be upgraded to include socio-economical, ecological, and geographical factors for a more generalized representation. At the same time, satellite imagery is being used for unsupervised clustering to identify high-risk areas based on the correlation between the image data and socio-economic data. In addition, the impact analysis will enable the development of better linkages between demographics and covid threat severity. This will enable policymakers to identify the more vulnerable portions of society. Thereafter once the optimal link between demographics and the covid threat is established the identified demographic metrics can be used to generate tools for assessment of current pandemic threat level from infection data features. The information extracted from the detailed interviews and the survey data will be used to tune the emulation engine to better emulate real-world conditions under various containment strategies and thereby estimate the socio-economic impact and effectiveness of containment measures more accurately. Then the preceding tasks will be aggregated to generate helpful information and insights related to the pandemic and socio-economic impact.

The intermediate outcomes of the AI models will be matched and validated in the field using experimental studies. Twenty individuals will be randomly selected for each study group to represent various age groups, socio-economic backgrounds, educational levels, etc. A control group and an experimental group will be constructed from the selected individuals, and the outcomes of the AI models will be applied to the experimental group.

Finally, the outcomes of the AI models will be mapped to actionable policies. Moreover, workshops will be conducted as the final task to effectively develop accessible outcomes for the policymakers and medical professionals.

Figure 1 shows data collection, processing and output approach based on the methodological approach described above.

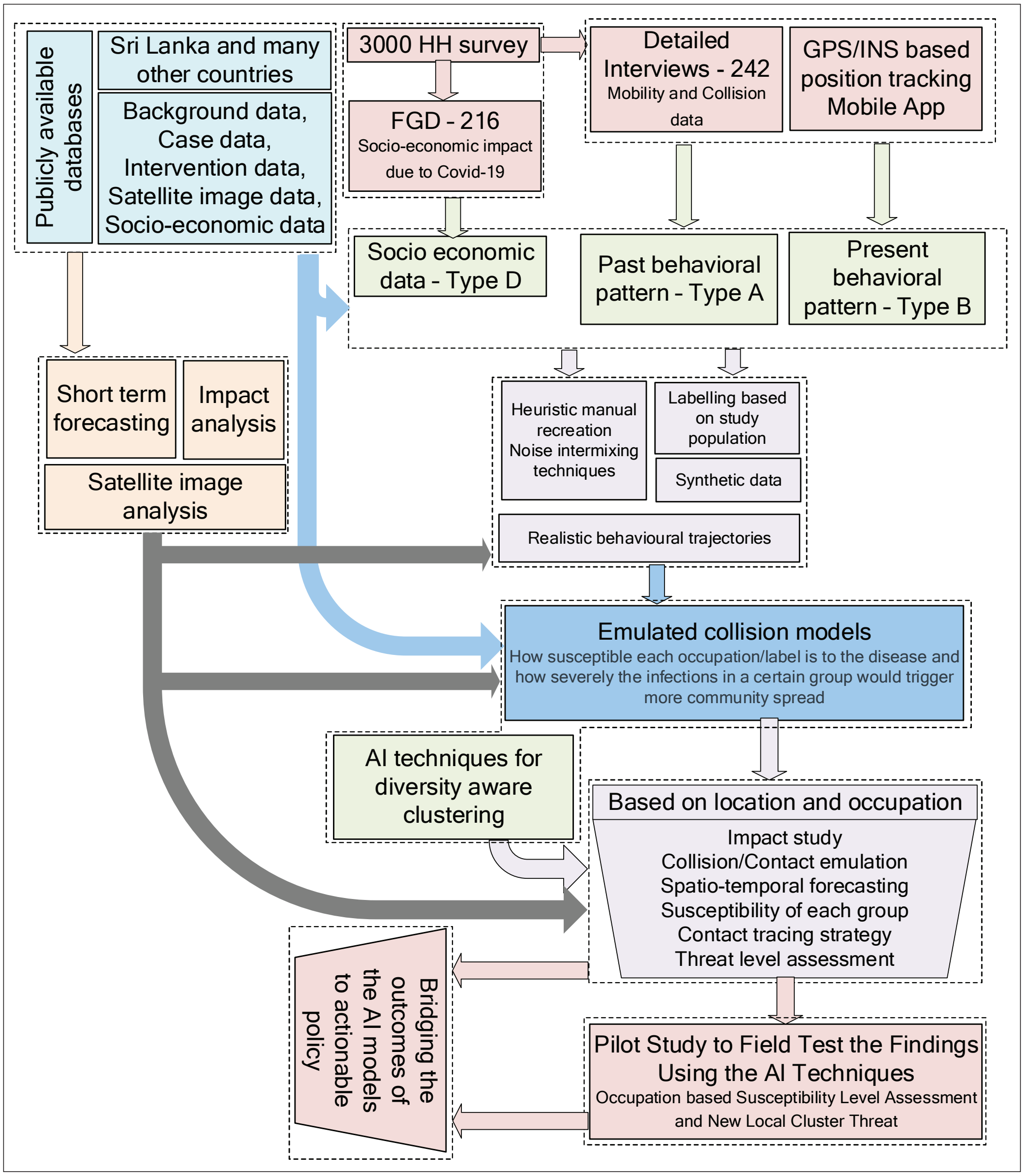

Figure 1: Methodological approach of this project

RESULTS AND DISCUSSION

Household survey

The massive socio-economic impact of COVID-19 has caused measurable and non-measurable changes to human lives. These impacts are diverse among different groups of people depending on their gender, income, ethnicity, culture and other social and economical aspects. In support of analysing these impacts and finding the best possible solutions to mitigate the adverse impacts, a large household survey was conducted in Sri Lanka. This survey was completed by the mid of December 2021 covering 3020 households and 12153 individuals. Data collection was conducted all around the country including 20 districts, 89 divisional secretariat divisions and 201 Grama-Niladhari divisions. These areas were selected purposively using reports by the Department of Census and Statistics, Epidemiology Unit, Ministry of Health, news bulletins, press releases and expertise knowledge. The high-risk areas were considered according to the risk maps by the Department of Census and Statistics. Further, major economic zones such as agricultural, fishing, estate and industrial were specifically selected using the past statistics of various issues of Central Bank and census reports. A total number of 15 households from each Grama-Niladhari division were sampled with the help of village-level administrative officers. Enumerators were well trained for interviews and all enumerators were undergraduates who were pursuing degrees related to social sciences at the time of data collection.

Preliminary qualitative results of the survey

Impacts on employment, education, health, income, food intake, mobility, cultural activities and socio-political aspects due to the COVID-19 pandemic were gathered from this survey. The basic demographic data and qualitative findings, especially the impacts of employment and education, are presented in this study. According to the descriptive statistics, the whole data set has a proper gender balance (female 57.3% and male 42.7%) and age distribution. The sample consists of diversified ethnicities including Sinhalese, Muslim, Tamil and mixed ethnicities. The education level of individuals ranged from no education to postgraduate level qualifications in the selected sample.

According to the qualitative results of the survey, most of the families were severely affected due to the pandemic either economically, socially, spiritually or culturally. Among them, the impact on income sources was severe. Containment strategies like travel restrictions, lockdowns and social distancing limited most of the employment, especially own businesses:

"I am a tourist driver. I lost my employment completely due to the pandemic and the restrictions imposed" (Male, aged 50, Kandy)

"I had a retail shop, and it is my household's main income source. Due to the pandemic I had to close the shop because public transportation was completely stopped" (Female, aged 48, Alawwa)

The education system was changed drastically due to the pandemic creating both positive and negative outcomes. According to the results, students' preference towards modes of education was diverse among different age groups and educational levels. Some students are still waiting for their usual classroom education while some of them highly prefer online education due to many reasons. Additionally, most of the students prefer a hybrid system of education which consist of both face-to-face and online classes:

"I prefer online education because it saves my time for transportation. Also, I can access classes from many teachers if needed. Therefore, online learning is far better than face-to-face education" (Female student, aged 17, Kaluthara)

"I missed being with my friend because of online education" (Male student, aged 15, Negambo)

"I prefer a hybrid system. I have household chores as a mother. Therefore, it will facilitate me to choose among the mode of education" (Female postgraduate student, aged 28, Gampaha)

Future work using the household data

This large survey data will facilitate the selection of specific groups of people such as students, informal and formal sector workers, elderly people, ethnic minors and village level administrative officers for Focus Group Discussions (FGDs). This task will collect data on the socio-economic impact of individually selected groups and create a broader picture of the impact of the pandemic. Furthermore, these data collected from the household survey and FGDs demonstrate the socio-economic impact of the pandemic on severely impacted groups in Sri Lanka at a high-resolution level. These data will be used to analyse further socio-economic impacts of the COVID-19 pandemic on identified diverse groups using social science research methods and to feed the emulation engine to produce outputs using AI-informed strategies.

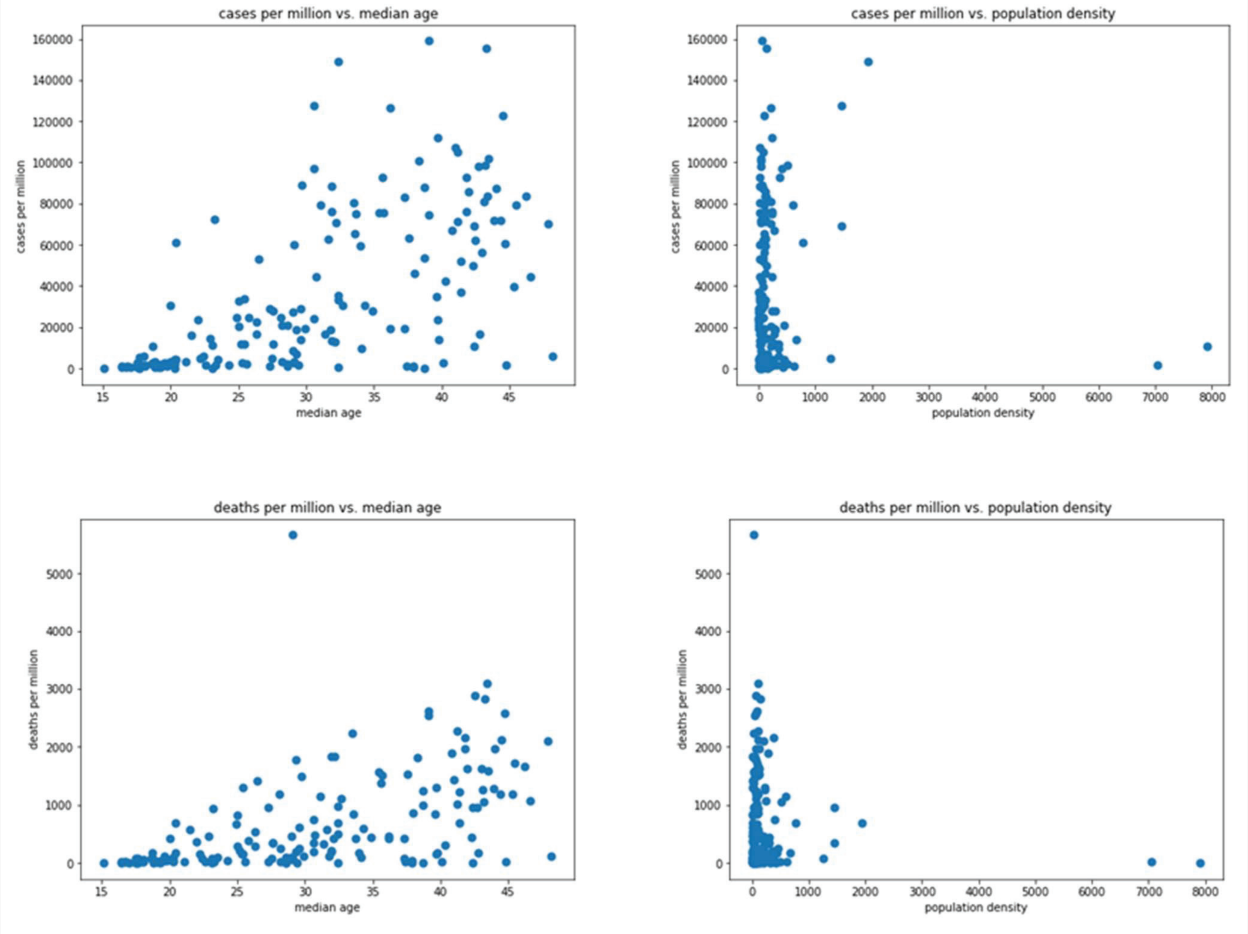

Figure 2: Correlation of the cumulative cases and deaths per million with median age and population density

Impact analysis

The demographic profile of a country is at play when it comes to the progression of COVID-19 as it spreads through contacts. The demographic profile (or population pyramid) provides cues about the general socio-economic status of the population of a country (Heenan, 1965; Peters *et al*., 2010). To study the effect of the demography on the cumulative cases and deaths of a country, prominent demographic parameters, median age and population density, were considered as the independent variables. The strength of the relationship between the independent and dependent variables was defined using the correlation coefficient between the variables. The correlation results are depicted in Figure 2 for cumulative cases and deaths under median age and population density.

According to the correlation study, it was observed that the median age was better correlated with the cumulative cases and deaths than population density as the independent variable. In particular, median age recorded a correlation coefficient of 0.6311 and 0.5876 with cases and deaths per million respectively. A correlation of this magnitude indicates that the variables are moderately correlated. On the other hand, population density had a correlation coefficient of -0.0107 and -0.1274 with cases and deaths per million respectively, which concludes that the variables are weakly correlated. This instigates that the severity in terms of normalized cases and deaths is better related to the age demographics of a country rather than the residency demographics.

However, median age only represents a cross-section of the age demographics of a country as the median age does not change with symmetric alterations to the population pyramid around the median age. Therefore, it is important to consider the entire population profile when assessing the relationship between the age demographics and cumulative normalized cases and deaths of the country for severity. For simplicity, the Bloomberg resilience index (Bloomberg, 2021), a numerical quantification of COVID-19 severity, was used to study the connection between the population pyramid to Covid severity.

For that, the population pyramid was clustered using the UMAP algorithm and the results are depicted in Table 1. The embedding algorithm maps countries with similar population pyramids to nearby locations and vice versa. According to the classification results, it was observed that, in general, countries with similar demographic profiles had similar resilience rankings which suggest that the total age demographics is a suitable candidate to study the Covid severity of a country. These impact studies enable a better understanding of the relationship between population demographics to pandemic threat for policymakers. These enable us to identify vulnerabilities in our population demographic spread. The next step would be to link infection time series data features to better assess current threat levels of the pandemic. This will help policymakers respond in proportion to the actual threat present.

Table 1: Clusters of countries according to demographic profile and respective resilience scores

Cluster 1	Cluster 2	Cluster 3	Cluster 4	Cluster 5	Cluster 6
Japan -14	Czech Republic-31	Norway-7	Israel-4	Sri Lanka-47	UAE-12
Italy-30	UK-11	Singapore-2	Argentina-53	Iran-48	Nigeria-37
Portugal-20	Canada-18	Russia-26	Brazil-51	Indonesia-42	Iraq-44
Greece-33	Poland-39	US-13	Vietnam-23	Malaysia-35	Pakistan-46
Germany-28	South Korea-5	New Zealand-1	Turkey-41	India-50	
Spain-17	Belgium-25	Australia-3	Colombia-52	Saudi Arabia-19	
	Romania-38	Tahiland-27	Peru-49		
	Denmark-8	Ireland-22			
	France-24	China-9			
	Switzerland-16	Chile-36			
	Netherlands-29				
	Hong Kong-10				
	Bangladesh-40				
	Austria-21				

Satellite image-based threat assessment

The basic idea in this task is to find a correlation, between data retrieved from satellite image clustering and demographic parameters. In particular, this task intended to identify low income dense urban-dwelling areas using spectral clustering techniques. This will help in assessing how much of a risk it is for a particular area to be a Covid hotspot. Satellite images of the city area of Kandy and Colombo were obtained in the resolution of 4318×3904 for this purpose. The satellite image area of Kandy is shown in Figure 3. Satellite images were clustered using spectral clustering and k-means clustering to see the best method that can be used for grouping data from satellite images. Figure 4 shows a result of clustering a small segment of the satellite image.

As the Grama-Niladhari division is the smallest division in Sri Lanka, this resolution was selected for demographic data collection. Land use, ethnicity, and industry sector data were collected from data available in the Department of Census and Statistics, Sri Lanka. According to the collected data, the relevant Grama-Niladhari division was clustered into groups. As the next step, we will be developing a correlation between the clusters from the satellite image processing and demographics data clustering. These enable us to develop visual identification tools for threat assessment and containment. It also helps the policymakers to prioritise response levels based on regional clusters to focus more energy on the more vulnerable zones.

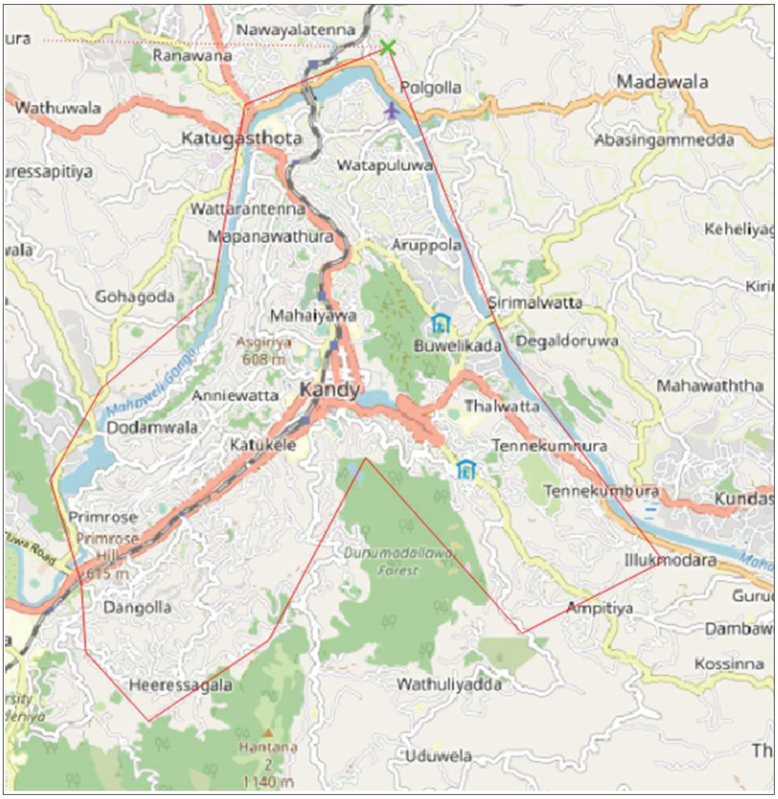

Figure 3: Study area of Kandy from the received satellite image

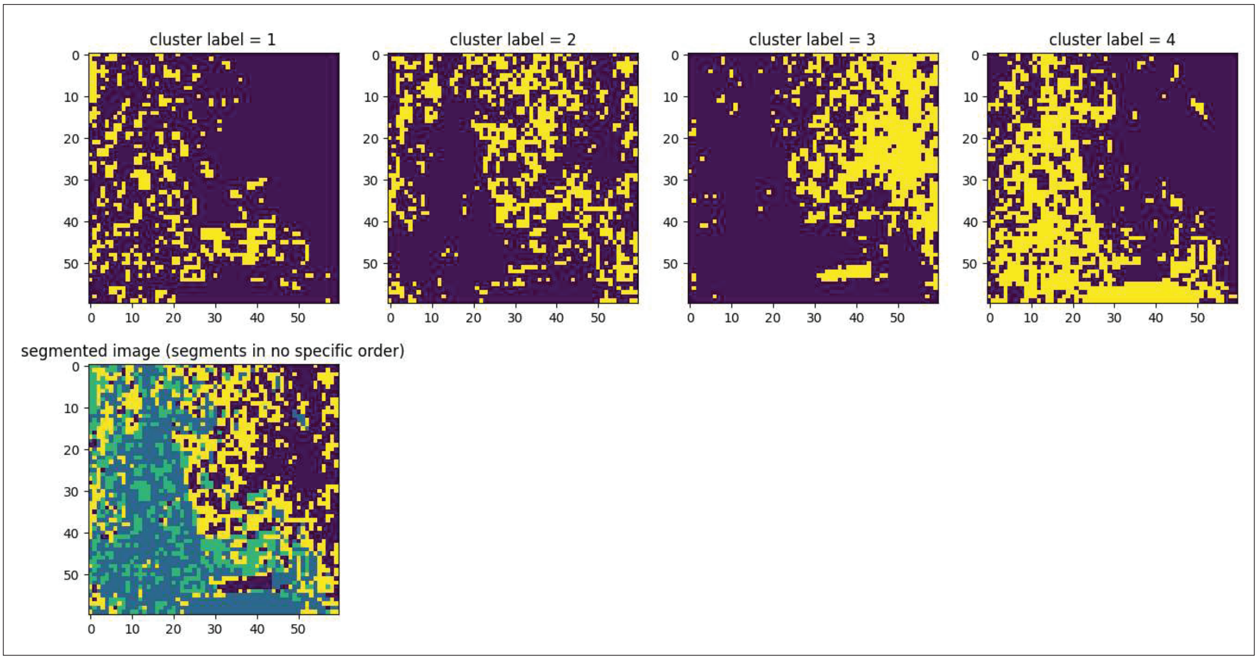

Figure 4: Individual clusters and the segmented image from satellite data of the city area of Kandy

COVID-19 forecasting

There are several compartment modelling methods like SIR, SIER models to analyse the spread of diseases. They construct mathematical relationships between each compartment using the number of people in each compartment and the rate of change of the people. The infection epi-curve of the model is fitted to real-world data by fine-tuning the parameters of these mathematical relationships to forecast future cases. However, these compartment models give the overall picture of the spread of the disease, and it does not identify the impact on each individual or group of people. Also, these compartment models forecast the number of cases for a long period and the number of cases in the immediate future (two weeks in advance) is not accurate. The objective of developing a forecasting model is to have accurate short-term predictions that would be useful in implementing containment measures at a country level or a regional level. This could help avoid or mitigate the adverse impacts on vulnerable groups due to actions taken using long term predictions.

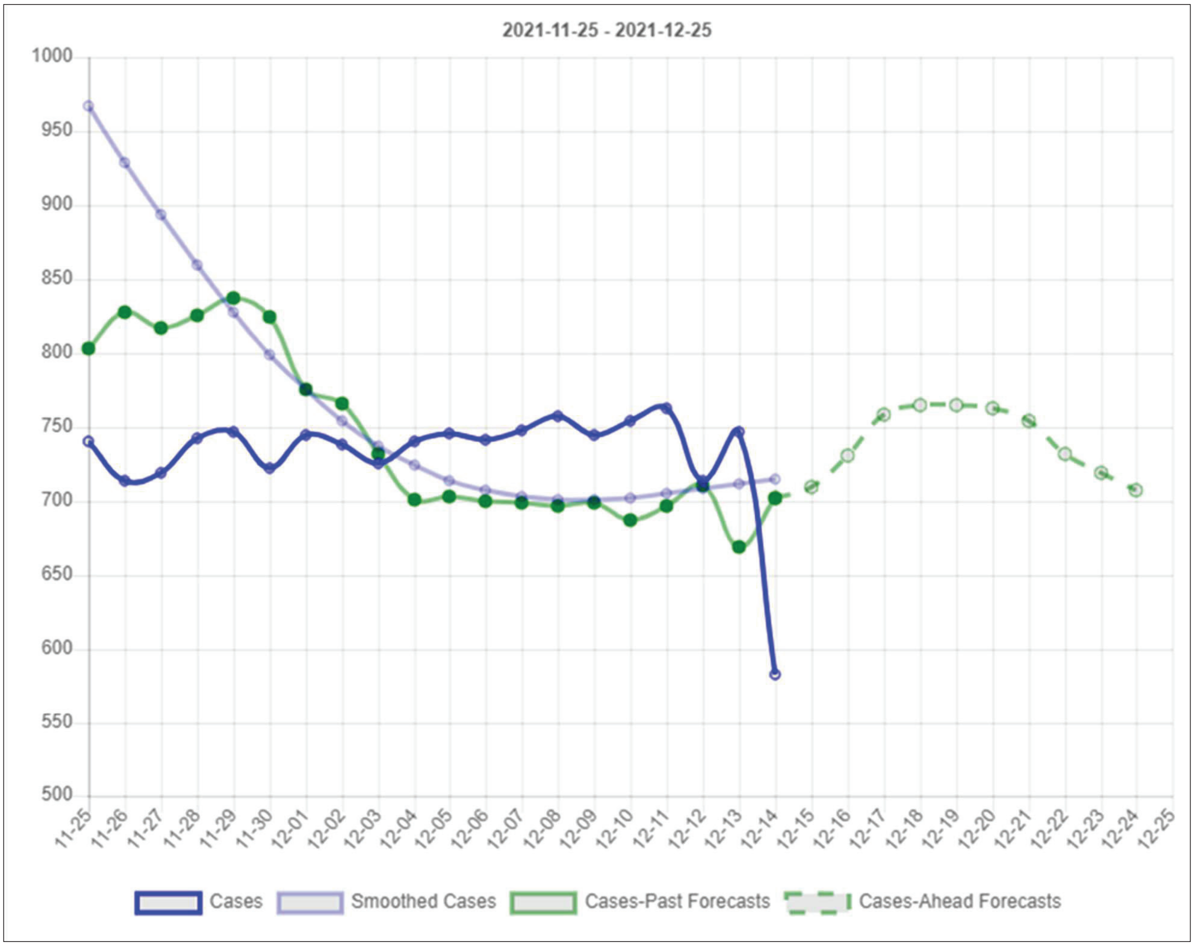

Figure 5: Two-week prediction of new COVID-19 cases in Sri Lanka

The data collected from many countries have a significant noise on each epi-curve of new COVID-19 cases because most of the countries have a varying number of tests each day. Also, some of the test results were released with a delay that makes the daily new cases higher than actual cases. To mitigate these issues a novel smoothing technique is presented which is adaptive to each epi-curve. The collected regional data from different countries were first smoothed using adaptive low pass cut-off frequency in the Fourier domain. Then these data were normalized and split into training and testing samples. These data were then trained using a Long-Short Term Memory (LSTM) neural network to predict the future new number of cases up to two weeks using 50 reported daily new cases. Since there is a lack of COVID-19 data for training, district-wise data and county-wise data from a diverse set of countries were used to train the data. The two-week prediction of Sri Lankan COVID-19 cases and the previous predictions were illustrated in Figure 5.

COVID-19 simulation

The simulator developed using an agent-based model uses data collected from the survey to simulate groups of people classified by their occupation to move around a virtual environment. The demographics of the simulated population of 300 is shown in Figure 6. These movements are adjusted in such a way that reflects real-life movements. The environment is modelled by implementing each location (Homes, Parks, Hospitals, Schools, Offices, etc) in a tree-like structure which gives hierarchical features to these locations. A simple example of the tree structure is shown in Figure 7.

The mobility of the agents is ingrained into the simulator using the probability of visiting a particular location during a given time of the day. Using this probability of visiting a location, assume that the simulator decides the daily routine of a particular agent is going to the park from home and then later coming back home after visiting a shop in the commercial zone. The movement can be simulated in the simulator as shown in Figure 7, where the agent first moves from the house to the park through the housing scheme. Then, the agent should move from the residential zone to the commercial zone to visit the shop. In the figure, the locations visited by an agent during the day are marked as A, B, and C. The path that the agent takes to traverse the tree is shown by a black line. Thus, using a hierarchical tree structure to model the environment is useful when simulating such complex movement patterns. Also, there are different transportation methods implemented in the simulator. For example, the agents will use public transport systems when travelling from residential zones to commercial zones. Also, when the agents are inside a building or a park, they will walk or ride using a bicycle.

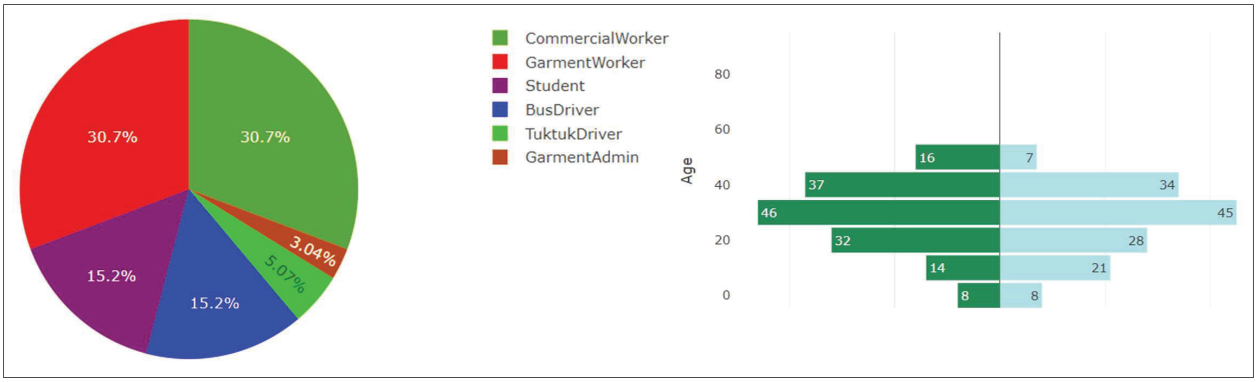

Figure 6: Occupation distribution and Population pyramid of the simulated population

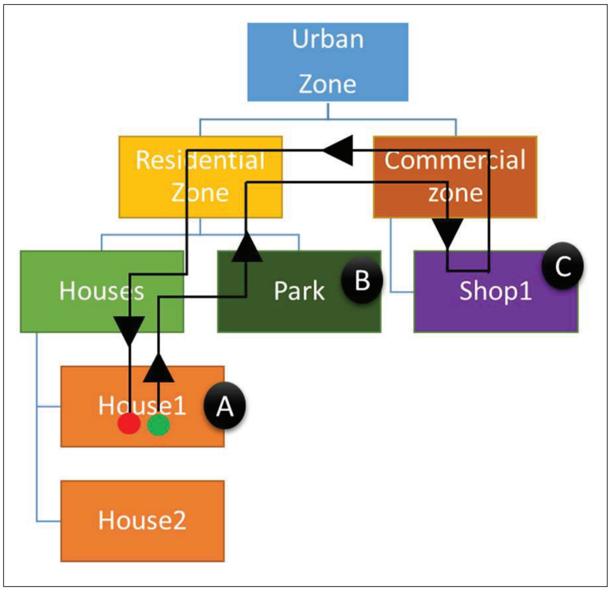

Figure 7: Hierarchical structure of a simple environment modelled in the simulator

The different states of each agent are logged to identify the spread of the disease. Also, when an agent is infected by another agent, the source of the infection, location of the infection is recorded to analyse the critical locations that spread the disease. Further, the people who are in contact with each other are also recorded to find the groups of people who are actively engaged with other people. Different states (Infected, Susceptible, Recovered, and Dead) of the population and other features like vaccination and testing information are illustrated in Figure 8.

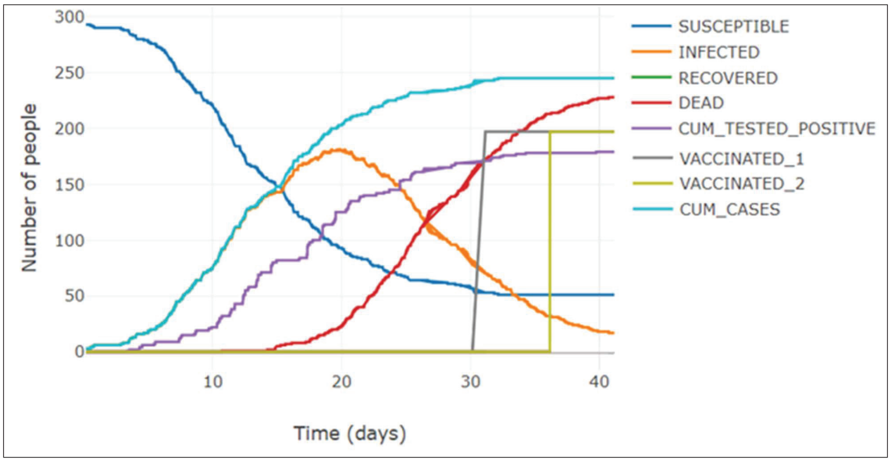

Figure 8: Variation of population states with time

Figure 8 reflects the normal compartment model distribution of states with time. But the ABM simulator is capable of identifying different vulnerable groups in detail and uses contact tracing to track the spread of the disease as well. Also, different testing and containment strategies can be employed to analyse the effect of those policies using the simulator.

CONCLUSION

COVID-19 caught the world off-guard. The world was unprepared for a pandemic of this magnitude and proportion, as was evident by the hasty response and reaction offered by nations and stakeholder organizations. Though many of the measures adopted were generally in “good faith” while considering the “best data and information available at the time”, and with the sole intention of reducing the spread of the disease (i.e. “flattening the curve”), they instigated numerous adverse socio-economic consequences.

In the face of the sudden rise in the number of cases and deaths due to COVID-19 as well as the high case-fatality rate and the high infectivity rates reported early on, prompted many countries to take various containment strategies ranging from open (no containment), social-distancing, restricted, lock-down, to complete shut-down. Especially, the intense containment strategies adopted by many countries had little or no consideration towards socio-economic ramifications of the said response or the impact on women, children, socio-economically underprivileged groups. The existence of many adverse impacts raises questions on the approaches taken and demands proper analysis, scrutiny, and rigorous review of the policies. Equipped with the wealth of data, lived experience, and diverse scenarios collected over the COVID-19 pandemic thus far, AI poses a viable paradigm for such scrutiny. In this study impact of these diverse containment measures was investigated using a survey while considering identified diverse groups such as women, children, and the socio-economically underprivileged groups. The survey data were collected from 12153 individuals, and they are now being processed to be fed into AI tools that are under development. The data will also be analysed quantitatively and qualitatively to draw immediate impact metrics for under-identified diverse groups.

Considering fact that the optimal containment is not only country/region-specific but also situation-specific, a proper understanding of region-specific demographics and how they link to covid threat levels is essential. Therefore, satellite imaging and the population pyramid will be linked to pandemic threats. In addition, accurate estimation of current covid threat level and predictions of threat for the near future is required for timely yet rapid response decisions making.

The core of the AI engine is under development to identify different vulnerable groups in detail and uses contact tracing to track the spread of the disease. This engine can be used to identify the origin of the disease spreader events, analyse the effect of different containment and testing strategies, track the groups of people who are frequently in contact and predict the severity of the outbreaks using the data from the real world.

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REVIEW

Interpretability and accessibility of machine learning in selected food processing, agriculture and health applications

N Ranasinghe[1], A Ramanan[2], S Fernando[3], PN Hameed[4], D Herath[5], T Malepathirana[1] , P Suganthan[6,7], M Niranjan[8] and S Halgamuge[1*]
[1] *Department of Mechanical Engineering, University of Melbourne, Australia.*
[2] *Department of Computer Science, Faculty of Science, University of Jaffna, Sri Lanka.*
[3] *Department of Computational Mathematics, Faculty of Information Technology, University of Moratuwa, Sri Lanka.*
[4] *Department of Computer Science, Faculty of Science, University of Ruhuna, Sri Lanka.*
[5] *Department of Computer Engineering, Faculty of Engineering, University of Peradeniya, Sri Lanka.*
[6] *KINDI Center for Computing Research, College of Engineering, Qatar University, Doha, Qatar.*
[7] *School of Electrical Electronic Engineering, Nanyang Technological University, Singapore.*
[8] *Department of Electronics and Computer Science, Faculty of Engineering and Physical Sciences, University of Southampton, United Kingdom.*

Summary: Artificial Intelligence (AI) and its data-centric branch of machine learning (ML) have greatly evolved over the last few decades. However, as AI is used increasingly in real world use cases, the importance of the interpretability of and accessibility to AI systems have become major research areas. The lack of interpretability of ML based systems is a major hindrance to widespread adoption of these powerful algorithms. This is due to many reasons including ethical and regulatory concerns, which have resulted in poorer adoption of ML in some areas. The recent past has seen a surge in research on interpretable ML. Generally, designing a ML system requires good domain understanding combined with expert knowledge. New techniques are emerging to improve ML accessibility through automated model design. This paper provides a review of the work done to improve interpretability and accessibility of machine learning in the context of global problems while also being relevant to developing countries. We review work under multiple levels of interpretability including scientific and mathematical interpretation, statistical interpretation and partial semantic interpretation. This review includes applications in three areas, namely food processing, agriculture and health.

Keywords: Disease detection in agriculture, drug repositioning, food processing, interpretation of neural networks, metagenomics.

INTRODUCTION

Artificial intelligence (AI) has seen an explosive growth over the last 20 years, largely through recent advances in machine learning (ML) – the data-centric branch of AI. A data-centric AI system consists of an AI model (a structure or architecture) and a method or learning algorithm that enables that model to derive usable information from data. Sometimes the data are exploratory, like the genomic data arriving from different parts of the world about constantly mutating viruses. To discover the presence of new variants or labels, we can feed an AI model with such uninterpreted data, so that researchers will be able to use this AI model to assign labels. Such AI models need unsupervised learning (UL) algorithms to extract information from unlabelled and uninterpreted data. We could also ask those researchers themselves to label the data with appropriate variant labels, and feed both labels and genomic data to an AI model that can then use a supervised learning algorithm like deep learning (DL), so that it can serve as a predictor for known variants of the virus. If such an AI model of sufficient strength requires it to be large, deep and complex, we call it a deep neural network (DNN). Shallow neural networks,

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[*] Corresponding author (saman@unimelb.edu.au; https://orcid.org/0000-0002-2536-4930)

commonly referred to as neural networks (NNs) are data driven mathematical models consisting of about three layers of artificial neurons or nodes (several linear and nonlinear processing elements) which are interconnected through weighted connections.

Popular models of AI, in particular, ML based models have significant deficiencies preventing its broader application. For example, they are mostly uninterpretable (Halgamuge, 2021). This paper addresses some of these in the context of several major global problems in the post-pandemic world with relevance to developing countries.

Achieving interpretability in ML will promote broader and effective use of ML, by answering core questions about traceability, accountability, ethical compliance, and inherent biases. Serious concerns remain about the reliability of ML systems in certain contexts. For example, the claim about achieving self-driving status for vehicles has not allayed public concerns. Explainable AI (XAI) methods can help in some applications although may not provide a full explanation about the decision process of the ML model.

There are two main strategies that exist for achieving higher accessibility through automated ML model design. In "growing the ML model from scratch" the ML model starts with a default simple model and grows until its capacity is enough to solve the problem. This approach has been used in both supervised and unsupervised learning-based ML systems. In the "search for the best among selected candidates" approach, also called neural architecture search (NAS), the best ML model is selected from a list of candidate solutions. The former is applicable to both labelled and unlabelled data, whereas the latter is generally confined to labelled data.

Most ML research on increasing interpretability of ML systems on the Y-axis and accessibility through increased AI model design automation on the X-axis (Figure 1) lies along or close to the axes. Research into interpretable neural networks designed with minimal expert intervention (FAIR AI) will increasingly close a significant knowledge gap, also informed by relevant studies, for example, ML with continuous and life-long learning capability (Senanayake *et al*., 2021).

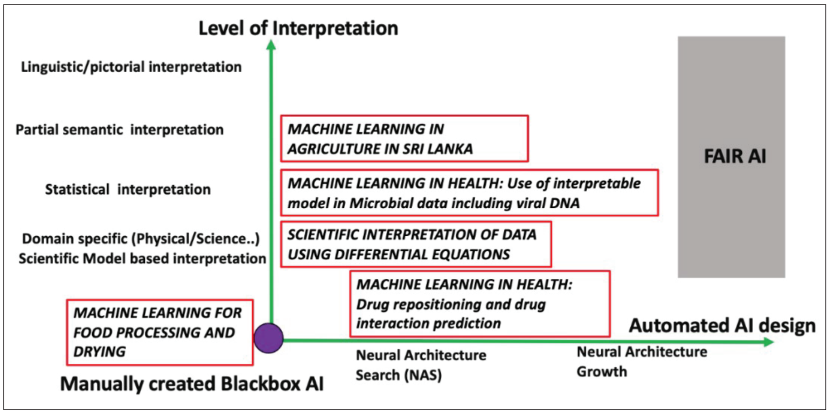

Figure 1: Contributions of the Paper and Fair, Accessible, Interpretable and Reproducible (FAIR) AI adapted from Halgamuge (2021)

This paper is organised with an example of scientific interpretation capability of ML models using differential equations followed by relevant applications of ML in three areas of importance, namely Food Processing, Agriculture and Health: examples of uninterpretable ML models applied to food drying, partially interpretable convolutional neural networks (CNN) and XAI in plant disease detection including in rice cultivation, safety of taking multiple pharmaceutical drugs and reuse of existing drugs for new diseases using semi-automated unsupervised ML model construction and shedding some light into yet largely unexplored world of microbes including viruses using semi-automated ML model construction.

SCIENTIFIC INTERPRETATION OF DATA USING DIFFERENTIAL EQUATIONS

Supervised learning problems generally focus on learning a relationship that maps a given input space to an output space based on input-output pairs. This involves training a model to learn this relationship by looking at a labelled dataset. Although most trained models including neural networks can be represented as a mathematical function, interpreting this function would typically be quite challenging. Only a very few models such as linear regression models will have an interpretable mathematical equation. CNNs are ML models with some (pictorial) interpretability. It can be noted that most equations modelling real-world problems do not have a very large number of terms (Brunton *et al*., 2016). Furthermore, differential equations (DE) are commonly seen as governing equations of dynamical systems. These DEs are generally derived using first principles. Recently there has been a focus on recovering governing differential equations from observation data of dynamical systems.

Udrescu and Tegmark (2020) propose a recursive multi-step method to search through the possible space of equations that fit a given dataset. It uses properties commonly found in real-world physics equations to reduce the search space. These include symmetry, separability, compositionality and more simplifying properties. This paper uses a neural network as a function approximator to discover some of these simplifying properties. The final equation is discovered using a brute force search across the simplified solution space. Udrescu *et al*. (2020) improve upon this approach to discover Pareto-optimal formulae (complexity vs accuracy).

Brunton *et al*. (2016) approach the same problem by using sparse regression techniques to circumvent the problem of searching through a large space of possible solutions. Here, the differential equation is assumed to be in the form of,

$$\frac{d}{dt}X(t) = f(X(t))$$

Where X is the independent variable and $f(X(t))$ is assumed to be a linear combination of non-linear functions of X. A library $\theta(x)$ of possible terms are constructed using these non-linear functions. The resultant formulation is,

$$\frac{d}{dt}X(t) = \theta(X)\mathrm{E}$$

Where, E includes the coefficients corresponding to each library term. The problem is now in the form of a linear regression problem. The paper uses a sparse regression algorithm to solve for the coefficients since real life differential equations typically do not have many terms in the right-hand side. This idea is improved and applied to learn partial differential equations (PDE) in Rudy *et al*. (2017). Zhang and Lin (2018) propose a method to use Bayesian sparse regression to increase the robustness of the learned equation and quantify the uncertainty of the solution.

Neural networks are also used in recent work to recover governing differential equations from observed data. Martius and Lampert (2016) used neural network algebra to learn equations through backpropagation. A shallow neural network is used with custom activation functions which include multiplications, trigonometric functions and identity functions. Sparsity promoting L1 regularization is used to promote learning a simple equation with a low number of terms. Sahoo *et al*. (2018) proposes a method to extend the class of the learnable equations using an equation learning network to include divisions. Long *et al*. (2019) propose a deep neural network architecture to discover time-dependent PDEs from observed data. This architecture proposes the use of a δ-block, which uses convolutions to approximate differential operators and a symbolic neural network to approximate the non-linear response function. The approximation framework is as follows:

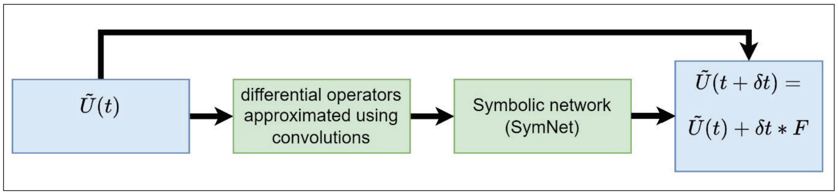

Figure 2: PDE-NET 2.0 (Long *et al*., 2019)

$$\widetilde{U}(t+\delta t) \approx \widetilde{U}(t) + \delta t * F$$

where, $\widetilde{U}(t)$ is the predicted value at time t and F is the PDE-NET approximation. The architecture of a δ-block is shown in Figure 2. A single-block can only approximate one-step dynamics, meaning that it is prone to error accumulation over time. Multiple-blocks with shared parameters are stacked to approximate multi-step dynamics and reduce error accumulation.

Most works in this area focus on learning a certain type of ordinary differential equation (ODE) or PDE. However, when applying these methods to real scenarios, we may not know the type of equation in advance. Furthermore, most of the approaches in the literature can only learn 1st order differential equations. Therefore, future work could focus on learning higher order DEs. Scalability is also a challenge in current methods and is especially evident in NN based equation learning algorithms. These are quite resource intensive and in certain cases require GPUs to run. Moreover, only a very few works explore methods of uncertainty quantification of the solution. This is also a potential research direction.

MACHINE LEARNING FOR FOOD PROCESSING AND DRYING

Food security is a major concern around the world as foods have high nutritional value and are an essential part of everyday life. However, most plant-based foods are highly perishable and about one-third of global food production is lost annually due to inadequate processing (Vilariño *et al*., 2017). This issue is amplified in developing countries where about 40% of seasonal fruits and vegetables are wasted (Karim & Hawlader, 2005). Drying is a major food preservation and processing technique which aims to remove moisture, preventing microbial spoilage (Kumar *et al*., 2015), thus adding value to a product, permitting early harvest, reducing shipping weights and costs, minimizing packaging requirements and increasing shelf-life (Zielinska *et al*., 2013). However, drying is highly complex involving interconnected simultaneous momentum, heat and mass transfer with time-varying physiochemical and anisotropic structural changes which depend on the dynamic product-drying environment interactions occurring (Welsh *et al*., 2018; Khan *et al*., 2020). Additionally, the underlying mechanisms, which originate within the materials cells, are still not well understood. Due to these complexities, optimising the design of dryers and process conditions is challenging and expensive. AI, for example, uninterpretable ML, has demonstrated great potential to facilitate innovation and optimisation for the processing of foods, providing a low-cost alternative to the current design modelling approaches and in-line process controllers. Converting these ML models to interpretable ML models will have significant advantages.

Uninterpretable Machine Learning

ML (e.g. NN) is able to investigate, model and predict the nonlinear time-varying behaviour of food material during drying (Angermueller *et al*., 2016). The three main parts of NN represent the input layer (material definition and/or process conditions), the hidden layer (consisting of nodes and weighted connections) and the output (drying rate, moisture content evolution and/or quality). The approach utilises experimental examples of the system which is being modelled (training data) to optimize the parameters including weights of the connections between neurons to predict the complex outputs. Over the years various NN models have been applied in food drying for predicting complex outputs for various materials (Sun *et al*., 2019) and various drying technologies (Sarkar *et al*., 2020). Generally, ML is applied to predict four types of outputs for food processing, the evolution of moisture and temperature, morphological/structural changes, transfer coefficients and quality changes.

Common complex outputs predicted through ML models include the moisture and temperature evolution of the material and the drying rate/transfer coefficients of a dryer. Predicting such complex outputs can provide insight into the drying time and the energy consumption of a dryer. Chasiotis *et al*. (2020) utilised a NN to predict the moisture content evolution of convective drying cylindrical quince slices. This work utilised 1372 experimental samples split between training data and cross validation data to consider three input variables (temperature, flow velocity and time) to predict the samples' moisture content. The results showed good agreement between the predicted and experimental values. Çerçi and Daş (2019) applied a NN and decision tree to predict the heat transfer coefficient for natural and force convection, concluding NN was more successful in estimating the heat transfer coefficient. Saraceno *et al*. (2012) investigated and compared three different modelling approaches, a thin-layer model, a NN and a hybrid neural model for two different vegetables with different characteristic dimensions for a wide range of process conditions. Their work demonstrated that pure neural models gave very accurate predictions when modelling/reproducing known data/process conditions. However, the accuracy of the NN model significantly decreased when attempting to extrapolate/apply the model to unknown scenarios/process conditions.

The morphological and quality changes food material experiences during drying have also been predicted through ML techniques. Scala *et al*. (2013) constructed a NN model for predicting the quality characteristics of Granny Smiths apples during convective dehydration. The work investigated experimental data from three different drying temperatures (40, 60 and 80°C) drying at three air flow rates (0.5, 1.0 and 1.5 m/s) and effectively predicted the colour, water capacity and total phenolic content of the samples. Additionally, the work identified the optimal drying conditions within the experimental process conditions. Chen and Martynenko (2013) utilised computer vision to evaluate the drying rate, shrinkage and colour changes of two varieties of blueberry, though the accuracy of the shrinkage measurements was limited by the pixel resolution of the camera and the accuracy of the colour measurement was limited by the quality of illumination and colour reproduction. Recently, Sinha and Bhargav (2022) developed an ANN model to predict key properties related to shrinkage, specifically solid density, initial porosity and initial water saturation of a given food material, using temperature and moisture data from a set of simple experiments. The work demonstrates how a NN model can serve as an efficient indirect method of property estimation in food material.

ML techniques have also been applied to calculate key transport properties. Mariani *et al*. (2008) developed an NN-based inverse method to estimate the apparent diffusivity of bananas at different drying temperatures. The work found that a small change in drying temperature and moisture content caused a significant change in bananas' apparent diffusivity. Sablani and Rahman (2003) developed an NN model for predicting the thermal conductivity of various foods in terms of moisture content, temperature and apparent porosity. NN models have demonstrated great predictive capabilities in comparison to other statistical approaches.

Although AI and ML techniques provide a low-cost alternative to facilitate innovation and optimisation for the processing of foods, applying ML to food processing does have some challenges. ANN is considered to be a 'black box' approach (i.e. uninterpretable) where the user cannot see what is happening during the simulation which is not ideal for understanding what is occurring during drying. Additionally, ML is a data-driven approach where the accuracy of the model heavily depends on acquiring a large comprehensive training dataset. However, food processing data is often scarce and obtaining large datasets is expensive due to the resources and costs involved in measuring this data experimentally. As a result, most NN models in literature have been constructed using small datasets and therefore their accuracy significantly decreases when applying the model to data outside the training observatory data (Saraceno *et al*., 2012). Recently, a new class of NN or deep learning, physics-informed neural networks (PINN), has emerged that can seamlessly integrate training data and complex mathematics to optimise a loss function. By incorporating prior knowledge, it minimises the need to have a large observatory training dataset for maintaining the high accuracy of the predictive model. Hence, overcoming the main challenges for AI in food processing. A PINN model can be trained with additional information obtained by enforcing physical laws with mathematical governing equations (Karniadakis *et al*., 2021). PINN models have been constructed for the deformation of elastic plates (Li *et al*., 2021), engineering heat transfer applications (Zobeiry & Humfeld, 2021), modelling fluid mechanics (Raissi *et al*., 2020), understanding permeability and viscoelastic modulus properties (Yin *et al*., 2021) and to solve forward and inverse problems (Zhang *et al*., 2019). Though demonstrating great potential, the PINN modelling approach has not been applied to food drying yet. For additional information on AI for the application of food processing, readers are directed to the comprehensive reviews of Sun *et al*. (2019), Khan *et al*. (2020) and Nayak *et al*. (2020). More insights into PINN modelling approaches can be found in Karniadakis *et al*. (2021).

MACHINE LEARNING IN AGRICULTURE IN SRI LANKA

Sri Lanka is a tropical country with a high potential for cultivating and processing a variety of crops. Although the open-field agricultural system is the most prominent in Sri Lanka, the sector is increasingly enabled by the recent growth of IoT applications and AI systems in the field. Greenhouse agriculture is an emerging subsector today that attempts to maximize the harvests under a protected cultivation environment within the limited space. The recent government restrictions on chemical fertilizers have regulated the local agriculture industry to look for optimal fertilizer combinations that maximize yield in open-field systems and green-house environments. As the research of different permutated fertilizer combinations increases, numerous plant diseases and nutrient deficiencies receive increased attention from ML researchers.

Rice is one of the most popular food crops in the World and one of the main grains used in Asia including in Sri Lanka. Jaffna is located in the Northern tip of Sri Lanka at a longitude of 79° 45′ – 80° 20′ and east latitude of 9° 30′ – 9° 50′ with a population of around 700,000. The agriculture and fishery sectors play a crucial role in the gross production of Jaffna. Since the soil and climatic conditions are favourable to cultivate a wide range of crops including paddy, agriculture plays a significant role in the lives of people in Northern Sri Lanka. Various diseases in paddy agriculture have been seriously affecting rice production and constitute a big challenge for the agricultural community to ensure food security. These diseases include rice blast, bacterial leaf blight, bacterial leaf streak, sheath blight, seedling blight, false smut, rice hispa, sheath rot, root knot, leaf streak, yellow stem borer, brown spot, and brown planthopper (Figure 3) mainly caused by viruses, bacteria, fungi, etc. Diseases of rice plants could be affected by different factors, such as fertilizers, nutrients, water management, climatic conditions, lighting conditions, humidity, and farming conditions. The detection of such rice plant diseases is normally performed on visual assessment of the symptoms which is subjective, time-consuming even for well-experienced experts and is prone to error. Automating such visual assessment will provide information for the prevention and control of rice diseases through which the quality and the quantity of rice production can be increased by reducing the operation costs. Thus, the automation will contribute significantly to the economic growth of Sri Lanka.

Explainable AI for plant disease classification

The diagnosis of plant nutrient deficiencies at early stages is essential as it indicates the impact of the applied fertilizer combination on plant growth. This deficiency could be a reason for overdose or lack of a particular nutrient or a combination. These deficiencies could be mainly seen in the plant's stems, leaves, or terminal buds (Figure 3). The association of these symptoms to different nutrient deficiencies requires expert knowledge. A data-centric approach is to use a classification model trained on labelled data. Image processing applications in the field of agriculture help to identify and diagnose crop diseases, pests and weeds, and the classification of agricultural products. CNN is one of the most effective methods of image processing which extracts image features, achieves high-level fusion of semantics and deep extraction of features through multilayer networks. Due to inherent learning capability, CNN is the prominently used machine learning model for plant diseases classification. The convolution operation identifies the local features and through the processing in the subsequent layers of CNN, it hierarchically develops motifs and parts that could represent the symptoms of a particular disease. Despite the high classification accuracy, the CNN architecture is further explored to shed some light into the explanation or interpretation of its decision making. While techniques such as temperature scaling (Guo *et al*., 2017) have been used to calibrate the CNN confidence scores, the explainable artificial intelligence models (XAI) have been hybridised with the CNN architectures (XAI-CNN) to provide explanations for such classifications. In general, these XAI models relate to or explain the class labelling by identifying the mostly contributed regions of the input image. The more commonly used XAI techniques are reviewed next emphasizing the main principle behind them.

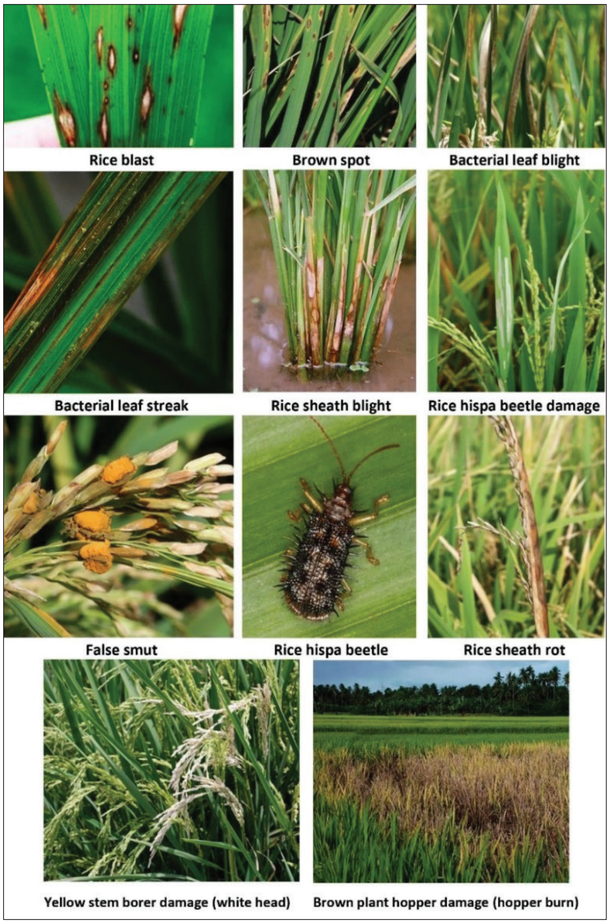
Rice blast
Brown spot
Bacterial leaf blight
Bacterial leaf streak
Rice sheath blight
Rice hispa beetle damage
False smut
Rice hispa beetle
Rice sheath rot
Yellow stem borer damage (white head)
Brown plant hopper damage (hopper burn)

Figure 3: Examples of common rice diseases (courtesy of *http://www.knowledgebank.irri.org/*)

Generally, XAI techniques that explain single disease predictions are of great interest in plant disease classification. CAM (Zhou *et al*., 2016), LIME (Ribeiro *et al*., 2016), and SHAP (Lundberg & Lee, 2017) are commonly used XAI models which provide justifications by generating local attention maps or by modelling feature importance. CAM (Class Activation Map) and its derivatives such as GRAD-CAM (Selvaraju *et al*., 2017) and GRAD-CAM++ (Chattopadhay *et al*., 2018) are visual explanation techniques that can identify discriminative image areas by locating the pixel groups responsible for influencing the association between a given input image and a particular class label. These pixels are determined by projecting classification layer weights or classification layer 'gradients of activation' to the convolution feature maps. In contrast, LIME (Local Interpretable Model - Agnostic Explanations) attempts to develop linear (interpretable) models over the complex perceptron model by identifying significant features of a data sample. It modifies a single data sample by tweaking the feature values (or identifying super-pixels) to understand the relationship between input and the predicted output. By doing so, LIME provides a list of explanations reflecting the contribution of each feature in the prediction of the disease class of a given data sample. In SHAP (Shapley Additive Explanations), the feature values of a data sample act as players in a coalition. Shapley value is the average marginal contribution of a feature value. These values calculate the feature importance and explain the association of the data sample to a particular class label. When applying these XAI models, several challenges are recognized. In LIME, it is hard to model non-linear relationships with linear models even by tweaking features or identifying the super-pixels. The perturbations of a single data point with random noises would not cover the variations in the dataset. In SHAP, the Shapley values provide additive contributions of explanatory features, which might be misleading if the model is not additive. Further, it is not mathematically feasible to calculate the Shapley values for all the classification problems. Therefore, mainly CAM-based XAI techniques have been applied in plant disease classification (Liu *et al*., 2020) (Ennadifi *et al*., 2020) demonstrating significant improvements in the explainability of the class-label predictions.

When employing CNN together with XAI models, few challenges are recognised. First, publicly available datasets are small and often imbalanced leading to models getting overfitted even if the data are augmented with various augmentation techniques. Therefore, when applying XAI techniques on small and imbalanced datasets, CAM and GRAD-CAM approaches do not perform well because it becomes difficult to locate the correct region of the convolution feature maps just by activating the classification layer of these models. This problem gets severe as the number of classes increases. Therefore, developing large balanced datasets is vital.

Second, a deficiency could be associated with multiple stages: early-stage, mild-stage, and severe stage, and symptoms of several deficiencies could be overlapped making the classification challenging. For example, there could be both Ca+ and B deficiency; their symptoms could not be separable at their early stages but separable when they reach the mild stage. Therefore, applying CNN models at different stages of the deficiency could result in different predictions with a low confidence score. Using XAI models on poorly calibrated models further decays the explanations' accuracy. Therefore, reasons based on feature activations or feature importance are insufficient for plant disease classification. It requires a much richer analysis of the features to map their activation to more probable two or three classes (including the predicted) based on the stages of the deficiency and growth stage of the plant.

Although existing XAI techniques give explanations in terms of feature activation and importance that can be interpreted by machine learning researchers they cannot fully explain the decision-making process in terms of agricultural technology and therefore of less use in real applications in disease recognition in agriculture. Therefore, these illustrations must map into human-understandable explanations for example by integrating the concepts of expert systems on the activated convolution features of XAI.

Rice disease detection using convolutional neural networks

Due to the inherent ability of the CNN model to show layer by layer pictorial explanation of how an input image is processed to reach the classification outcome, it is a good candidate to use in detecting rice diseases even without hybridizing with XAI.

Jiang *et al*. (2020) proposed a rice disease recognition model that combines CNN and support vector machine (SVM) to identify four types of rice diseases. Images were pre-processed by applying mean-shift algorithm to segment lesions from rice leaf disease. From the images of leaf lesions shape features such as area, roundness and shape complexity were extracted, and the colour features were extracted using CNNs by converting RGB colour space into HSI and YCbCr colour space. The extracted features were then classified by an SVM with RBF kernel due to the small number of sample images. They collected a total of 8911 images from rural farmland and from rice leaf disease atlas in which 6637 images of disease rice were manually cropped. A classification rate of 96.8% was reported.

Anandhan and Singh (2021) proposed a rice disease recognition model by comparing Mask R-CNN and Faster R-CNN algorithms to identify five types of rice diseases. Images were pre-processed using a multistage median filter to reduce noise. They implemented the model using ResNet-50 (Residual Neural Network) as the backbone and optimised the model using a stochastic gradient descent optimiser. In each iteration, a mini batch of size 60 was used. The learning rate was set to 0.01 with weight decay fixed to 0.0001. They collected about 1500 images from rice plants in the region under different weather conditions and images were manually annotated for diseases. A classification rate of 87.5% was reported for Mask R-CNN which outperforms Faster R-CNN.

Liang *et al*. (2019) proposed a rice blast recognition method using CNN. In their work, high-level features extracted by CNN were compared with handcrafted features: Harr-wavelet Transform and local binary patterns histograms (LBPH) in rice blast recognition. In addition to using CNN in the recognition task, the extracted features were also classified by SVM using an RBF kernel. Moreover, they created a rice blast disease image set that is made publicly available (*http://www.51agritech.com/zdataset.data.zip*). Images encompassing 2902 negative and 2906 positive samples were considered for testing and training a CNN. Quantitative analysis results show that CNN with Softmax and CNN with SVM have almost the same performance. A classification rate of 95.83% was reported for CNN showing better performance for the said recognition task.

Zhou *et al*. (2019) proposed an approach to identify three types of rice diseases based on FCM-KM and Faster R-CNN fusion techniques. Images were pre-processed using a weighted multilevel median filter for noise removal. Thereafter, Otsu threshold segmentation algorithm (Otsu, 1979) was applied to segment lesions from leaf disease images. In order to extract rice disease characteristics, the FCM-KM was used to reset the bounding box size in Faster R-CNN for the convergence to be accelerated. The optimal value of K in K-means was selected with the maximum and minimum distance algorithm and where those initial cluster centres should be positioned. In addition, the dynamic population firefly algorithm based on the chaos theory was applied to the clustering process to jump out of the local optimum and obtain faster convergence. The FCM-KM and Faster R-CNN were applied to extract the disease characteristics and classify the images for pests and diseases. An image set consisting of 3010 images was tested and a classification rate of 97.2% was reported for the proposed method.

Sreevallabhadev (2020) proposed a method to identify rice blast disease by using CNN for feature extraction and SVM for classification. Experiments were carried out with 60 experimental configurations that vary in the choice of deep learning architecture, training mechanism, and dataset type. They used approximately 60,000 images of plant leaves in their original dataset (*https://www.kaggle.com/abdallahalidev/plantvillage-dataset*) and created three different versions of the dataset by considering colour, grayscale and leaf segmented images. An AlexNet based on CNN was used in extracting the features that are then fed to an SVM classifier. A classification rate of 96.8% was reported.

It is worth noting that, a majority of the work reported in the literature uses the following experimental setups and points out that there is a huge room for carrying out research in this domain using deep learning approaches.

- Evaluate on small scale datasets consisting of 500 to 5000 images beyond the work in (Sreevallabhadev, 2020). The small sized datasets directly impact the quality of the mapping function approximated by neural networks.
- Develop models to recognise a few rice diseases though there are several known diseases that greatly affect the quality and quantity of rice production.
- Propose methods mainly using features such as shape, texture and colour by the use of histogram-oriented gradient (HOG), scale-invariant feature transform (SIFT), wavelet, local binary pattern (LBP), HSI and YCbCr, CNN, etc.

MACHINE LEARNING IN HEALTH

Drug repositioning and drug interaction

Drug repositioning and drug interaction prediction are two fundamental applications of drug development and clinical care that have significant benefits in pharmacology. Repositioning of existing drugs can be classified as single drug-based repositioning and drug combination-based repositioning. Drug combinational treatments are identified to be much effective for treating some diseases. Moreover, drug-drug interactions (DDIs) are likely to occur when a pair of drugs or a combination of drugs are co-administered. Investigating harmful DDIs is essential to enhance the effects of clinical care.

In these contexts, the features of existing drugs, such as chemical structures, gene expressions, target proteins, side effects, indications, etc., are considered to compare the pairwise drug similarity (Sun *et al*., 2016; Hameed *et al*., 2017; 2018) Further, chemical structural data, gene expression data, side effect related data and the transcriptional responses have been used for predicting drug mechanism of action (MoA), i.e., the prediction of molecular targets for a particular drug. 'Connectivity Map' (Lamb *et al*., 2006) resource, which is frequently used in related studies, can be used to find connections among small molecules sharing an MoA, chemicals and physiological processes, and diseases and drugs.

Over the past two decades, machine learning and network analysis-based drug repositioning have gained popularity. Machine learning approaches incorporate clustering, classification and deep learning techniques with statistical concepts, whereas network analysis approaches represent pharmacological knowledge as networks with nodes and edges. In network-based analysis, features such as genes, proteins, molecules and phenotypes can be used as the nodes. Their functional similarities, mode of actions and relationships can be represented by the edges which interconnect the nodes.

In network-based analysis, techniques such as random walk (Zhang *et al*., 2017), node embedding (Su *et al*., 2021), matrix perturbation (Zhang *et al*., 2017), and Steiner-tree based algorithms (Sun *et al*., 2016) were used. They focus on building a network using drug features, actions, and characteristics to predict the novel drug-repositioning candidates via drug-drug, disease-disease, and drug-disease relationships. Zhu *et al*. (2020) proposed a drug-centric graph model, extracted and integrated six drug knowledge bases and constructed the drug knowledge graph. They have used a path-based data representation method and embedding-based data representation for comprehensive analysis for drug repositioning. Both methods applied to the drug knowledge graph evidenced better predictive performance on diabetes mellitus treatments. In matrix perturbation, novel drug-disease prediction can be transformed into a missing link prediction problem. In Sun *et al*. (2016), the prize-collecting Steiner Tree approach has proven to be a promising subnetwork identification method for drug repositioning. Their Physarum-inspired subnetwork identification algorithm employed on drug similarity networks has inferred useful repositioning candidates for cardiovascular diseases.

Positive Unlabelled Learning (PUL) is an emerging topic in the field of computational drug repositioning and drug interaction prediction. The application of PUL for single-drug repositioning (Mordelet & Vert, 2011; Yang *et al*., 2012) and DDI prediction (Hameed *et al*., 2017; Zheng *et al*., 2019) has shown improved predictive performance. PUL enables prioritizing plausible negatives from the unlabelled data and improves performance compared to randomly selecting negatives from the unlabelled data. Mordelet and Vert (2011) introduced a scoring function and assigned a specific score to each data pair through which the data are sorted in descending order to distinguish positives and negatives. Similarly, Yang *et al*. (2012) have classified unlabelled data as reliable negatives, likely positives, likely negatives and weakly negatives. Hameed *et al*. (2017) also identified likely negatives from the unlabelled DDI pairs to treat as negatives when developing the binary classification models. These PUL approaches have shown significant improvements in final predictions.

Drug combination-based repositioning is emerging research in computational drug repositioning. Some recent studies focused on computational drug combination-based repositioning, exploring both therapeutic uses and adverse effects of drug combinations. Since there exist approximately 16,000 approved drugs on the market (Wishart *et al*., 2018), millions of drug combinations can be formed. However, only a small number of drug combinations is confirmed as useful with experimental research. Therefore, there is a need for accurate and reliable approaches to infer repositioning candidates from those millions of unlabelled drug combinations. PUL can produce significant improvements in drug combination-based repositioning as well. Further, awareness of harmful DDIs is beneficial to extract the most suitable drug combinations for drug repositioning.

Imbalanced data is another challenge arising in the drug repositioning and drug interaction prediction domains. Mostly balanced training sets are preferred in machine learning approaches and in model training which can improve the generalizability of the trained model by preventing the model from being biased towards a particular class. Wei *et al*. (2013) discovered that regardless of the rates of positives and negatives in human genome data, support vector machines trained on balanced datasets have performed well in binary classification. Leevy *et al*. (2018) have also emphasized the issues that may arise with high-class imbalanced datasets and have presented the possible solutions that can be applied to avoid such potential issues. Further, Hameed *et al*. (2017) emphasized using balanced datasets and employed multiple balanced training sets to strengthen the final prediction using ensemble learning which can reduce the variance of the final outputs.

Use of interpretable models in microbial data including viral DNA

Microbiology is the study of the structure and functions of microorganisms and their interactions with other microorganisms, species, or environments (Moitinho-Silva *et al*., 2017; DiMucci *et al*., 2018; Xie *et al*., 2019). Due to the advancements in microbial sequencing technologies, for instance, 16S rRNA sequencing, microbial studies generate a massive amount of data with a large number of samples and variables. Since most microorganisms are not present in most samples, the microbial data are also known to be highly sparse (Martino *et al*., 2019), adding to its challenges. Therefore, ML has been employed as a powerful tool that can analyse and identify significant patterns in microbial communities (Ghannam & Techtmann, 2021).

ML algorithms can appear in many forms in microbial data analysis. Dimensionality reduction and visualization leading to better interpretation of data is one such significant manifestation. To better comprehend the underlying patterns in the high dimensional microbial data, it is necessary to reduce the number of dimensions either to visually interpretable two or three dimensions or to a reasonable number of dimensions that can be used as the input to another ML model. Principal component analysis (PCA) (Wold *et al*., 1987) and principal coordinate analysis (PCoA) (Kruskal & Joseph, 1978) are two techniques commonly used for the task. However, they fail to capture the highly non-linear relationships in some microbiome data (Xu *et al*., 2016). In contrast, manifold learning techniques such as Isomap (Tenenbaum *et al*., 2000), Locally Linear Embedding (LLE) (Roweis & Saul, 2000), and t-Distributed Stochastic Non-linear Embedding (t-SNE) (Van der Maaten & Hinton, 2008) can overcome this limitation and therefore are commonly used in exploratory data visualization. t-SNE was also considered regularly for data visualization in the recent past on account of its capability in revealing the local structure in high-dimensional data, also bringing its attention to microbiome data (Kostic *et al*., 2015). Moving forward, Xu *et al*. (2020) proposed a t-SNE based classification method for compositional microbiome data using Aitchison distances as the conditional probabilities. By this method, authors were able to achieve better classification performance compared with the classifiers built in the original high-dimensional space. However, t-SNE's ability in preserving the global structure of data has been questioned. Therefore, a recently proposed technique, Uniform Manifold Approximation and Projection (UMAP) (McInnes *et al*., 2018) has gained popularity due to its capability in preserving the local structure while being superior in capturing the global structure compared to t-SNE. UMAP's usefulness in revealing composite patterns in microbiome data is demonstrated by Armstrong *et al*. (2021) with their application of the algorithm on three different microbial datasets. Autoencoders are artificial neural network-based algorithms that learn a compressed representation of the high dimensional input by minimizing its reconstruction error. Recently, autoencoders and their variations (e.g. variational autoencoders) have demonstrated their usefulness in learning meaningful latent features from data (Wang *et al*., 2014), showing potential to be used with microbial data. Therefore, the research in this domain would benefit from the concept of interpretable neural networks when optimally identifying a suited architecture for a particular problem and mapping the process of identifying latent patterns to human-understandable explanations.

Metagenomics which is the analysis of DNA sequences of multiple species is effectively being used for studying microbes, especially viruses (Herath *et al*., 2017). A metagenomic sample would consist of a large number of DNA sequence reads of multiple species making it inherently complex to study. Sample preparation including the DNA sequencing, reads assembly, annotation, and analysis of the data can be identified as the key steps in a metagenomic experiment and ML models are being used in all the mentioned steps (Krause *et al*., 2020). Metagenomic data can be used to infer the microbes' interactions with their host environments, and thereby are considered in microbiome biomarker discovery for disease diagnosis and monitoring. However, due to their complex nature, using metagenomic data in precision medicine as a decision support system demands interpretable models with conciseness and readability by non-experts (Prifti *et al*., 2020). Predomics, an ML approach inspired by microbial ecosystem interactions attempts to develop an interpretable and accurate model that can be used to analyse any type of data; especially microbiome data. Its effectiveness has been demonstrated on liver cirrhosis data (Prifti *et al*., 2020). Furthermore, the effectiveness of an interpretable ML approach has been demonstrated by a meta-analysis of 1042 fecal metagenomic samples. It suggests the use of interpretable models to obtain non-intrusive predictive disease biomarkers for colorectal cancer using metagenomics data of the gut microbiome (Casimiro-Soriguer *et al*., 2022). Furthermore, another recent study suggests that easily accessible microbiome samples and their metagenomic analysis using interpretable models have the potential for non-invasive diagnosis of diseases (Carrieri *et al*., 2021). The mentioned study demonstrates it by generating explanations for the predictions of skin hydration, age, menopausal status, and smoking habits based on leg skin microbiome (Carrieri *et al*., 2021). As such, as the sequencing cost is becoming low, low and middle-income countries can benefit from interpretable models for metagenomics data analysis that can be used for disease surveillance and anti-viral therapy.

DISCUSSION

Achieving interpretability in AI will promote broader and effective use of AI, by answering core questions about traceability, accountability, ethical compliance, and inherent biases. Several applications of AI reviewed in the paper provide different levels of interpretability from non to explainable AI. Interpretability can be further expanded to achieve linguistic interpretation (e.g. Halgamuge, 1997; Cao *et al*., 2020).

AI models have narrow accessibility as they are generally manually designed by experienced AI experts, which limits access for fields in which AI experts have no knowledge or interest. Some automation in the design of AI is used in the drug repositioning and drug interaction prediction application described. Other AI methods, for example, evolutionary algorithms, can be used to design AI systems automatically (Cao *et al*., 2020). There is vast untapped potential: for medical breakthroughs, distribution and use of scarce resources, forecasting with unheard-of accuracy in natural and economic domains, etc, when both interpretability and accessibility of AI systems can be combined as shown for small scale neural networks in Halgamuge (1997).

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REVIEW

Mosquito control: A review on the past, present and future strategies

SHPP Karunaratne[1*] **and SN Surendran**[2]
[1] *Department of Zoology, Faculty of Science, University of Peradeniya, Peradeniya, Sri Lanka.*
[2] *Department of Zoology, Faculty of Science, University of Jaffna, Jaffna, Sri Lanka.*

Summary: Nearly half a million deaths occur worldwide annually due to mosquito-borne diseases. Mosquito control has become the major strategy in controlling these diseases, especially in the absence of effective vaccines for disease prevention. At the beginning of the last century, mosquito control was mainly done by personal protection methods and larval control by application of petroleum oil and Paris green powder to water bodies. A breakthrough in mosquito control came in the 1940s with the introduction of synthetic neurotoxic insecticides which could suppress mosquito populations rapidly throughout the globe. However, a resurgence of populations with resistance to these insecticides was witnessed within a decade after their introduction. Environmental pollution caused by synthetic insecticides also became a major concern. Novel personal protection methods, community-level operations on source reduction, insect growth regulators and polystyrene beads for larval control, and biological control were introduced as alternatives. Biological control was mainly by larval predators such as fish, dragonfly nymphs, microcrustaceans and *Toxorhynchites* larvae; bacterial larvicides such as *Bti*; plant-based mosquitocides; and green-fabricated nanoparticles. However, even today, mosquito control programmes heavily depend on synthetic neurotoxic insecticides applied through insecticide residual spraying (IRS), fogging, larviciding and impregnated bed nets. Increased detoxification and target site insensitivity, developed as major insecticide resistance mechanisms, have been extensively studied in mosquitoes assisting proper management of available insecticides for which not many alternatives are available.

Despite all our efforts, an unprecedented global emergence of mosquito-borne diseases is evident demanding novel strategies for mosquito control. The introduction of transgenic strains of mosquitoes to suppress or replace mosquito populations reducing disease transmission has become the latest effort. Population reduction has been achieved *via* releasing mosquitoes with a dominant lethal gene (RIDL) and by combining the conventional sterile insect technique (SIT) with *Wolbachia* mediated incompatible insect technique (IIT). Population replacement has been successful *via* releasing *Wolbachia* infected mosquitoes that are refractory to pathogen development and transmission. Advancement of gene- and allelic- drive systems will soon allow us to effectively spread refractory genes and insecticide susceptible alleles into mosquito populations overriding normal inheritance.

Keywords: Biological control, insect growth regulators, insecticides, mosquito control, mosquito-borne diseases, transgenic mosquitoes.

INTRODUCTION

Until the late 1880s, malaria was thought to be a disease caused by bad air coming from swamps and rivers (the term malaria derived from the Italian ‘mal-aria’ or ‘bad air’). Malaria parasite was identified by Sir Alphonse Laveran in 1880, and in 1897, Sir Ronald Ross found the malaria parasite in the gut of an anopheline mosquito revealing that it is a mosquito-borne disease. Towards the end of the nineteenth century, it was established that certain species of insects, other arthropods and freshwater snails act as vectors of some important diseases (Rozendaal, 1997). Since then, the control of vectors became the main strategy in the control of transmission of vector-borne diseases, especially in the absence of effective vaccines for the prevention of these diseases.

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[*] Corresponding author (shppk@pdn.ac.lk; https://orcid.org/0000-0002-2537-0548)

Vector-borne diseases account for more than 17% of all infectious diseases, causing more than 700,000 deaths annually and a majority of these deaths occur due to mosquito-borne diseases such as malaria (causes more than 400,000 deaths every year) and dengue (more than 40,000 deaths annually) (WHO, 2020).

Mosquitoes belong to the Family Culicidae of the Order Diptera. The family consists of more than 3500 extant mosquito species which are distributed throughout tropical and temperate regions and beyond the arctic circle. Apart from being a biting nuisance, some mosquito species transmit deadly pathogens. Species that act as vectors of diseases are mainly included in the Genera *Anopheles*, *Culex, Aedes* and *Mansonia* (Harbach, 2013). Pathogens transmitted by mosquitoes include protozoans (e.g., malaria parasites), nematodes (e.g., filarial worms) and viruses (e.g., dengue, chikungunya, yellow fever, zeka, Japanese encephalitis, West Nile fever, Rift Valley fever). Effective mosquito control interventions are vital for the suppression of mosquito-borne diseases. Given the diversity of mosquito species, their biology and the diseases they transmit, different intervention strategies have been implemented across many regions globally and the selection of appropriate interventions for a particular vector to be controlled is of paramount importance. These interventions can be operated at personal level, community level or at government level.

PERSONAL PROTECTION TO AVOID CONTACT WITH MOSQUITOES

Avoiding or not visiting risky places during peak mosquito biting hours is recommended as a strategy to control mosquito bites. Hour before sunrise and hour after sunset are the peak biting periods for most mosquito species. Although there are diurnal biting mosquito species like *Aedes* spp., many prefer nocturnal biting between sunset and sunrise. Steps should be taken to close the windows and doors which are open to outside, in the early evenings. Host-seeking is odour-driven, and finding a host depends on the quality of the odour plume and the obstacle free route towards the host. Therefore, proper house design is effective in preventing mosquito house entry. At the end of the nineteenth century, the Italian malariologist Angelo Celli recognized malaria as a disease of rural poverty and demonstrated the effectiveness of house modifications in reducing mosquito entry (Celli, 1900). Most malaria infections in sub-Saharan Africa are acquired indoors through the mosquitoes predominantly entering houses *via* open eaves. Closing the eaves has effectively reduced vector entry in thatched houses (Jatta *et al*., 2018). Having a ceiling with an appropriate material can also close the mosquito routes *via* eaves. The gaps in walls associated with mud and grass construction, instead of bricks, are a greater determinant of mosquito vector entry (Ippolito *et al*., 2017). A significant protection to inhabitants can be given by making houses and shelters insect proof while allowing a good ventilation. Anti-mosquito screenings with cotton netting, metal/plastic screens or insecticide treated screens can be used for this purpose. Also, raised buildings have reduced malaria transmission in Africa. Since most malaria mosquitoes fly less than 1 m from the ground, raising buildings off the ground has prevented the entry of *Anopheles gambiae*, the principal African malaria vector. Mosquito house entry declined with increasing height, with a hut at 3 m reducing *An. gambiae* house entry by 84% when compared with huts on the ground (Carrasco-Tenezaca *et al*., 2021).

In southwest Ethiopia, people and cattle live in proximity and the presence of calves within and close to human dwellings has acted to draw malaria mosquitoes toward the human occupants increasing the risk of malaria. Hence, deployment of cattle far from human residence has been recommended to reduce human exposure (Zeru *et al*., 2020). Where malaria is transmitted by zoophilic vectors, two types of malaria control strategies have been proposed based on animals; using livestock to divert the vector (zooprophylaxis) or using livestock as baits to attract vectors to insecticide sources (insecticide-treated livestock). It has been recommended to use insecticide-treated livestock as an intervention in integrated control efforts for malaria and livestock diseases which are transmitted by moderately zoophilic vectors (Franco *et al*., 2014). It is important to consider the direction of winds before constructing a house close to a large water body or a marshy land because the wind can directly bring in the adults emerging from the water body (Rozendaal, 1997). A diversion can be made by putting up an animal hut like cattle shed or a piggery in between the water body and the house to divert mosquitoes to animals. However, keeping piggeries near paddy fields in close association with human settlements worsened the transmission of Japanese encephalitis in Anuradhapura, Sri Lanka in the late 1980s, since the pig acts as the amplifying host for the virus (Peiris *et al*., 1992).

Bed nets and indoor residual spraying (IRS) of insecticides have a high impact on reducing malaria transmission indoors. Long-lasting insecticide-treated nets (LLINs) have added advantages by having the repellent action. Because of this, mosquitoes do not enter through the holes or edges of the net and also they do not bite the net touching body parts from the outside through the net material (Curtis, 1992; Rozendaal, 1997). In order to get protection from outdoor mosquito bites, various personal protection methods are available and these act by providing a physical barrier between the vector and host, or by repelling or killing the vector. Long-sleeved shirts and long trousers are worn to limit the area of exposed skin. Permethrin-treated clothing is protective against a wide range of mosquito species and has been successfully used by commercial company workers and militaries for decades (Banks *et al*., 2014). Topical repellents, such as DEET (*N,N*-diethyl-*m*-toluamide), various derivatives of eucalyptus extracts [eg. para-menthane-3,8-diol (PMD)], can be used effectively in disease control. Volatile pyrethroid-based mosquito coils are commonly used to repel mosquitoes in indoors and portable mosquito coils have been highly recommended against outdoor biting mosquitoes (Tangena *et al*., 2018). Ultrasonic sound waves in the range of 20−70 kHz which are well above the upper human audible limit produce enough stress on the mosquito nervous system to repel them. Experiments have shown that mosquitoes are repelled significantly by ultrasonic emission in the ranges of 40−55 kHz frequencies (Ikeri *et al*., 2017).

SOURCE REDUCTION (PREVENTION OF MOSQUITO BREEDING)

The term source reduction refers to any measure that prevents the breeding of mosquitoes. Mosquito species differ in their preferences for breeding habitats. Thus, some species breed in clean water containers in and around houses, whereas others prefer polluted water in sanitation systems, man-made and natural habitats in rural areas, or even brackish water. It is important to study the exact nature of breeding habitats of a target species before implementing the intervention. Methods to control larvae include eliminating or changing the breeding places to make them unsuitable for the development of larvae, making breeding places inaccessible to adult mosquitoes, releasing fish or other predators that feed on larvae, and applying larvicides (Rozendaal, 1997).

Source reduction can be permanently achieved by altering or eliminating breeding places e.g., covering or screening water containers, draining ponds and marshes, filling in ditches and pools etc. This is known as `Environmental Modification'. `Environmental Manipulation' refers to semi-permanent measures e.g., cleaning up containers, clearing vegetation, flushing streams and repairing drains. In contrast to environmental modification, methods of environmental manipulation have to be repeated to remain effective e.g., water-level fluctuation, flushing (stream sluicing), changes in water salinity, shading of stream banks (used against larvae of mosquito species such as *An. maculatus* and *An. minimus*), clearing of vegetation (against larvae that do not prefer direct sunlight e.g., *An. balabacensis*). Removal of water plants is used to control larvae and pupae of the vector of brugian filariasis *Mansonia* spp., that pierce submerged parts of water plants with their respiratory siphons to reach air spaces within the plant tissues for breathing (Chang, 2002). Also, clearing water plants can increase the exposure of mosquito larvae to larvivorous fish and, to water currents and wave movement that would flush them. Straightening and steepening of shorelines of streams prevent formation of small water pockets for breeding.

Aedes aegypti and *Ae. albopictus* prefer to lay eggs in discarded receptacles such as used tins, pots, cups, bottles, tyres and coconut husks etc. Promising results have been obtained through awareness programmes followed by garbage cleaning at community level to eradicate these breeding grounds. Badly designed drainage and sewage disposal systems, and water-holding vegetation such as ornamental plants and pineapple plantations can also provide breeding grounds, especially for *Aedes* mosquitoes. Good planning, maintenance and law enforcement can drastically reduce mosquito breeding in these places controlling the disease (Sim *et al*., 2020). Chemical insecticides or other larvicidal agents such as oils or biological agents can also be applied to mosquito breeding sites, especially for places where habitat elimination may not be possible eg. pineapple vegetation, ornamental ponds.

Mosquitoes that breed in irrigation water can be controlled through careful water management systems. Tree species such as *Eucalyptus* that grow rapidly and have higher transpiration rates are used for drying marshy lands and other areas with high water tables to prevent mosquito breeding (Rozendaal, 1997). Expanded polystyrene beads that are non-toxic to humans and other organisms can be spread on the water as a measure to control mosquito larvae. The floating layer thus formed acts as a barrier between the water and air, and mosquito larvae die because they cannot reach the water surface to breathe. The beads do not decay and remain floating for years but are vulnerable to flushing. They have been successfully used to control mosquito breeding in abandoned gem pits in Sri Lanka and the expanded beads with a 2 mm diameter were the most effective for suffocating *Anopheles* larvae and pupae (Yapabandara & Curtis, 2002).

Towards the end of the 19th century, petroleum oil was heavily used to prevent mosquito breeding for malaria control. Application of the oil to water bodies makes a thin oil layer over the water surface interfering with larval breathing. From 1921 to the 1940s, Paris green, a green powder of copper acetoarsenite, was also adopted for the same purpose. Powder particles float on the water surface kill the surface feeding anopheline larvae (Rozendaal, 1997). Since the introduction of organochlorines as larvicides in the 1940s, application of synthetic insecticides became a major method in mosquito larval control.

USE OF SYNTHETIC INSECTICIDES

Today, vector control programs largely depend on the use of synthetic insecticides, especially during disease outbreaks. There are several classes of vector control insecticides; organochlorines, organophosphates, carbamates, pyrethroids, bacterial larvicides, insect growth regulators and novel classes such as neonicotinoids, spinosyns and pyrroles. The first four classes listed are referred to as "conventional" insecticide classes. Most classes have broad-spectrum effects on other organisms while bacterial larvicides and insect growth regulators are more specific to targeted organisms (WHO, 2021).

Conventional insecticides

Commonly used synthetic insecticides can be divided into 4 major groups: Organochlorines, Organophosphates, Carbamates and Pyrethroids. The organochlorine DDT was first discovered in 1939 and introduced as an insecticide useful in the control of malaria, yellow fever and many other insect-borne diseases in the 1940s. Organophosphates came to known as neurotoxins in the 1930s but their synthesis in large quantities as pesticides came after World War II. The popularity of organophosphates increased due to environmental concerns and resistance development against organochlorines in the 1960s. Although the insecticidal activity of carbamates was discovered in 1931, large quantities were synthesized for vector control purposes in the late 1950s (Kuhr & Dorough, 1976). Pyrethroids are analogs of the pyrethrum extracted from *chrysanthemum* flowers that were long known to have insecticidal properties. The first-generation pyrethroids were developed in the 1960s (e.g. bioallethrin, tetramethrin, resmethrin) and the second-generation pyrethroids (*e.g.* permethrin, cypermethrin and deltamethrin) in the 1970s. Unlike first-generation compounds, second-generation ones are more resistant to degradation by light and air. The target site of organophosphates and carbamates is the insect acetylcholinesterase which hydrolyses the neurotransmitter acetylcholine on the post-synaptic nerve membrane. Inhibition of acetylcholinesterase by the insecticides results in continuous stimulation of post-synaptic nerve membrane leading to the death of the insect. For pyrethroids and a group of organochlorines (DDT + its analogs) the target site is the voltage gated Na^+ channel (VGSC) regulatory proteins of the nerve membrane. The binding of these insecticides prevents closing of VGSC hindering the active outside pumping of sodium ions. This leads to continuous firing of the nerves causing the ultimate coma and death of the insect. For the rest of the organochlorines (cyclodienes), the target site is γ- aminobutyric acid (GABA) receptors which regulate Cl^- conductance through the nerve membrane. Binding to GABA receptors will also cause continuous firing of the nervous system leading to the death of the insect (Karunaratne, 1998).

Pyrethroids are safer since they have low mammalian toxicity. Both organophosphates and carbamates have shorter residual life. Organochlorines have high persistence in the environment accumulating toxicity through the food chains. Although organochlorines are banned in several countries due to environmental concerns, they are still heavily in use especially in African countries. During the period 2010–2019, the annual global amount of insecticides used for disease vector control was 3314 metric tonnes (t) of organochlorines, 1625 t of organophosphates, 677 t of carbamates and 194 t of pyrethroids. These insecticides were mainly used for residual spraying, followed by space spraying, larviciding and treatment of nets (WHO, 2021).

Insect growth regulators

Insect growth regulators (IGRs) are a diverse group of chemical compounds that are highly active on pre-imaginal stages of insects and the mortality occurs many days after the treatment. This is indeed a desirable feature of a control agent because the larvae of mosquitoes are an important source of food for fish and wildlife (Mulla, 1995).

The first account on the potential use of IGRs in insect control was in 1956, when juvenile hormone (JH) was isolated from the abdominal crude extract of the moth *Hyalophora cecropia* (L.). Later, it was established that topical application of the hormone prevents insect metamorphosis (Tunaz & Uygun, 2004). According to their mode of action, IGRs can be divided into two groups; Chitin synthesis inhibitors (CSIs) and Insect hormone analogs. Larvae treated with CSIs develop until molting but fail to ecdyse due to inhibition of the synthesis of the new cuticle. The second group includes substances that mimic the action of insect hormones. Molting hormone (ecdysone) is responsible for cellular programming and, together with juvenile hormone (JH) it initiates the molting process. When JH levels are high, the epidermis is programmed for a larval molt, otherwise, the epidermis is programmed for metamorphosis. Thus, application of JH analogs suppresses pupation (Tunaz & Uygun, 2004). During the period 2010–2019, the annual global amount (metric tonnes of the active ingredient - t) of IGR insecticides used for disease vector control were 0.1 t methoprene (JH analog), 0.6 t novaluron (CSI), 9.2 t pyriproxyfen (JH analog) and 9.9 t diflubenzeron (CSI) (WHO, 2021).

Neonicotinoids, pyrroles and spinosyns

Neonicotinoids were developed through the 1980s and 1990s and, similar to pyrethroids, have a lower mammalian toxicity. They selectively bind and interact with the insect nicotinic acetylcholine receptors (nAChRs) which are ligand-gated ion channels that mediate fast synaptic transmission in the insect nervous system. During the period 2010–2019, the annual global amount (active ingredient) of neonicotinoids used for disease vector control was 36 metric tonnes (WHO, 2021).

Spinosyns are a large family of unprecedented compounds produced in the fermentation of two species of *Saccharopolyspora*. They have a unique mode of action involving disruption of nicotinic acetylcholine receptors. When compared with many other insecticides, the spinosyns generally show greater selectivity towards target insects and lesser activity against many beneficial predators as well as mammals and other aquatic and avian animals (Kirst, 2010). Their insecticidal spectrum, unique mode of action and lower environmental effect make them useful new agents for modern vector control programs. The annual global amount (active ingredient) of spinosyns used for disease vector control during 2010-2019 was 16 metric tons (WHO, 2021).

Pyrroles (e.g., chlorfenapyr) are a broad-spectrum class of insecticides new to vector control. They are pro-insecticides that require initial activation by insect monooxygenases to produce the active compound. Unlike most of the other classes of insecticides, the site of action of pyrroles is not the insect nervous system and therefore shows no cross-resistance to mechanisms that confer resistance to standard neurotoxic insecticides. They act at the cellular level and disrupt respiratory pathways and proton gradients through the uncoupling of oxidative phosphorylation in mitochondria (Oxborough *et al*., 2015).

INSECTICIDE RESISTANCE IN MOSQUITOES

The development of resistance against commonly used insecticides is a major threat to mosquito control programmes in a situation where not many alternatives are available. Therefore, research on insecticide resistance, molecular mechanisms which underlie the resistance and rational resistance management are of paramount importance in controlling the development and spread of insecticide resistance in vector populations. Insects develop resistance against insecticides through two major mechanisms; Metabolic resistance (changes in insect enzyme systems for rapid detoxification of insecticides) and Target-site insensitivity (alterations of the target sites to prevent their binding to insecticides) (Figure 1). Reduced penetration (cuticular resistance), behavioural changes and increased excretion may also act as minor mechanisms (Karunaratne, 1998; Hemingway *et al*., 2004; Karunaratne *et al*., 2018).

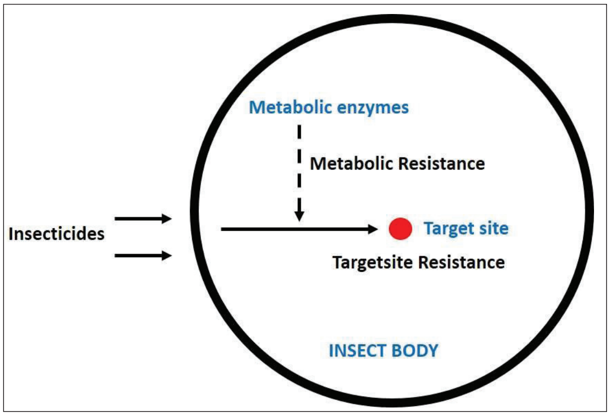


Figure 1: Mechanisms of insecticide resistance. Insecticides that enter the insect body can be detoxified by metabolic enzymes or can be prevented from binding to target sites due to structural alterations in target sites.

Three major groups of enzymes i.e*.,* esterases (also known as carboxylesterases), glutathione S-transferases (GSTs) and monooxygenases (also known as mixed function oxidases or cytochrome P450s) are known to be involved in insecticide detoxification in the insect body. Enhancement of enzyme activity in resistant insects compared to susceptible insects can be due to quantitative and/or qualitative changes of the enzymes in resistant insects. Increased amounts (quantitative changes) are produced in resistant insects as a result of gene amplification or increased upregulation of the gene. Increased catalytic centre activity (qualitative changes) is achieved through coding sequence mutations (Karunaratne *et al*., 2018).

Quantitatively changed esterase-based resistance mechanism has been studied extensively at biochemical and molecular levels in *Culex quinquefasciatus* and *Cx tritaeniorhynchus* mosquitoes. Esterases act by rapid binding and slow turning over of the insecticide. They sequester rather than rapidly metabolize the insecticide. Therefore, in order to keep the system effective, large quantities are required. It was found that the esterase gene is amplified up to 80 copies in resistant mosquitoes for the increased production of esterases (Karunaratne *et al*., 1993; 1998; Hemingway & Karunaratne, 1998). Increased esterase activity may not always be a result of increased quantity of the enzyme found in the resistant mosquito. A number of *Anopheles* species have a non-elevated esterase mechanism that confers resistance, specifically to malathion, through an increased rate of metabolism rather than through increased quantities. These esterases are called malathion carboxylesterases and have a higher catalytic center activity towards malathion than their susceptible counterparts (Karunaratne & Hemingway, 2001).

Resistance to insecticides can be provided by GSTs through several different pathways such as O-dealkylation or O-dearylation conjugation for organophosphates, dehydrochlorination and GSH conjugation for organochlorines, detoxification of lipid peroxidation products and passive sequestration for pyrethroids (Che-Mendoza *et al.,* 2009). Glutathione S-transferases are a diverse family of enzymes with more than 30 GST genes in mosquitoes. Additional diversity is contributed by alternative splicing to produce GSTs with differing substrate specificities (Ranson & Hemingway, 2005). In addition to conferring resistance *via* direct metabolism or sequestration of chemicals, GSTs also provide protection against oxidative stress-induced by insecticide exposure (Pavlidi *et al*., 2018).

Cytochrome P450 monooxygenases are also a complex family of enzymes that bind to molecular oxygen and receive electrons from NADPH to introduce an oxygen molecule into the substrate. Despite their ability to detoxify most of the insecticides of all classes, they mediate bio-activation of organophosphates by converting the `thionate' analog to more toxic `oxon' analog (Karunaratne *et al*., 2018).

Insects acquire target site insensitivity mainly through non-silent point mutations within structural genes. However, only a limited number of changes can decrease insecticide sensitivity without disrupting the normal physiological functions of the target site, Therefore, the number of possible amino acid substitutions is very limited. Hence, identical resistance-associated mutations are commonly found across highly diverged taxa (Karunaratne *et al*., 2018). Out of the two *ace* genes which encode different acetylcholinesterase proteins in insects, several resistance-associated point mutations have been identified in *ace-1* gene in insect pests including mosquitoes (Guo *et al*., 2017). `*knock-down resistance'* or `*kdr*' type gene mutations at *VGSC* gene prevent the target site binding to DDT and pyrethroids. More than thirty unique resistance associated mutations or combinations of mutations have been detected in pyrethroid and DDT resistant insect populations (Rinkevich *et al.,* 2013). `Resistance to dieldrin' (a cyclodiene organochlorine insecticide) or `*Rdl'* type alanine-to-serine (A296S) mutation is associated with GABA target site insensitivity in several species of dieldrin and fipronil in resistant mosquitoes (Yang *et al*., 2017).

In order to identify detoxification genes associated with resistance to insecticides, microarrays containing unique oligonucleotide probes for these genes have been constructed for *Ae. aegypti* and their expression level in insecticide resistant and susceptible strains have been compared. This '*Ae. aegypti* Detox Chip' has facilitated implementation of insecticide resistance management strategies (Strode *et al*., 2008). Increased activity of metabolic enzymes (esterases, GSTs and monooxygenases) can also be detected by conducting biochemical assays. Since acetylcholinesterase is an enzyme, its insensitivity to insecticides can also be tested biochemically by an inhibition assay (WHO, 1998). Various molecular techniques are now available to detect target site mutations involved in resistance (Karunaratne *et al*., 2018).

BIOLOGICAL CONTROL

Because of the development of resistance and the environmental pollution caused by conventional insecticides, biological control has always been welcomed as an alternative. Scientists had paid attention to use the prey-predator relationship in the environment for effective mosquito control from the very beginning. For this purpose, natural enemies such as aquatic insects, micro-crustaceans, fish and amphibians that prey upon mosquito immature forms have been extensively tested.

Additionally, microorganisms and their derivatives including bacterial, and botanical larvicides have also been developed.

Use of natural enemies as biological control agents

Aquatic insect species of the Orders Odonata, Coleoptera, Diptera and Hemiptera have the ability to prey upon mosquito preimaginal stages. Water bugs and immature stages of odonates are considered as voracious feeders on mosquito larvae (Shaalan & Canyon, 2009). In a study carried out in Sri Lanka, predatory efficacy of nymphs of five dragonfly species i.e., *Anax indicus*, *Gynacantha dravida*, *Orthetrum sabina sabina*, *Pantala flavescens* and *Tholymis tillarga* were tested against *Ae. aegypti* larvae and the highest predation rates were observed in *Anax indicus* followed by *Pantala flavescens*. The latter has been recommended as the best potential biological agent to control dengue vectors in the field considering its wider distribution and notable predation (Samanmali *et al*., 2018).

Mosquito larvae belong to the genus *Toxorhynchites* and, certain species of *Armigeres* and *Culex* prey upon other mosquito larvae (Surendran *et al*., 2013; Chathuranga *et al*., 2020; Donald *et al*., 2020). Adult females of the genus *Toxorhynchite*s are autogenous and do not require a blood meal for egg development. Although the prevalence of *Toxorhynchites* mosquitoes is mainly confined to forested areas in nature, they have been successfully reared in laboratories in large numbers and released to the environment to reduced dengue vector populations (Wijesinghe *et al*., 2009). Laboratory experiments have shown that *Tx. splendens* has preferential predation against *Ae. aegypti* larvae compared to other mosquito vector species such as *Ae. albopictus* and *An. sinensis* (Zuharah *et al*., 2015). However, *Toxorhynchites* as a biocontrol agent in the field has limitations due to its lower fecundity, prolonged larval development and the risk of cannibalism among own offspring (Donnald *et al*., 2020).

Microcrustaceans such as copepods are predators of immature forms of mosquitoes. Predatory efficacy of cyclopoid copepods on larvae of vector mosquitoes has been investigated in detail (Roa *et al*., 2002; Udayanga *et al*., 2019). A study carried out in Sri Lanka tested the feeding efficacy of five copepods namely *Mesocyclop leuckarati*, *Mesocyclops scrassus*, *Cyclops vernals*, *Cyclops varicans* and *Cyclops languides* on dengue vectors. The copepod *M. leukarati* has shown the highest predatory efficacy for both *Ae. aegypti* and *Ae. albopictus* (Udayanga *et al*., 2019). However, introduction of microcrustaceans to all possible *Aedes* breeding sites has been a severe limitation for its application (Hales & Panhuis, 2005).

Use of larvivorous fish as a mosquito control strategy in different aquatic habitats has been documented from many countries. Even though predatory efficacy of several different fish species on mosquito larvae has been reported, species belonging to the genus *Gambusia* and *Poecilia* are widely used as biological control agents against mosquito larvae in the field. *Poecilia reticulata* (guppy) is widely distributed in tropics and has a very high reproductive rate. Because of their small size they can creep into micro-habitats created especially by the root systems of aquatic plants. Also, as surface feeders they have a high opportunity in capturing mosquito larvae that regularly visit the water surface for breathing. These are ideal control measures for small water tanks and small ornamental garden ponds where the application of chemical larvicides needs to be avoided. Alternatively, bottom feeders like *Lepidocephalichthys thermalis* (common spiny loach) have shown negligible larvivorous potential since their swimming movements disturb the settled larvae. It has also been reported that column feeders like *Oreochromis mossambicus* (mossambique tilapia) show a moderate larvivorous potential (Ekanayake *et al*., 2007). Surendran *et al*. (2008) have also recommended *O. mossambicus* as a suitable biological control agent to control *Aedes* and *Anopheles* larvae. *Gambusia* sp. (mosquito fish) are surface feeders and are popularly used in mosquito larval control in several countries because of their greater efficacy on predation (Benelli *et al*., 2017). However, their presence has been considered to have a negative impact on the native biodiversity of aquatic systems as they become invasive and prey upon native fish species and amphibians (Mischke *et al*., 2016). *Poecilia reticulata* (guppies) and *Aplochcheilus dayi* (nalahandaya) have been recommended as the best candidates for biological control of *Aedes* larvae in Sri Lanka (Ranathunge *et al*., 2021).

Although the biological control of mosquito larvae using vertebrates has been mainly associated with fish, the potentiality of amphibians in this regard has also been documented. Predatory capacity of the tadpoles of frogs and toads on mosquito larvae has been studied under different conditions (Bowatte *et al*., 2013). Direct observations have substantiated that five tadpole species *Polypedates*, *Bufo*, *Ramanella*, *Euphlyctis* and *Hoplobatrachus* are *Aedes* egg predators under laboratory conditions. With about seven thousand frog species worldwide, living in a diversity of aquatic habitats where fish cannot reach, the role of tadpoles in the biological control of mosquitoes can be more significant than is currently understood (Bowatte *et al*., 2013). However, it has been suggested that tadpoles are generally herbivores and their predatory behaviour is merely due to competition since both mosquito larvae and tadpoles are detritus feeders (Weterings, 2015).

Use of microorganisms and their derivatives as biological control agents

Naturally occurring pathogenic microorganisms such as fungi, protozoans, viruses and bacteria can also be considered as potential agents for mosquito biological control. Toxins derived from some of these microbial agents are popularly used as alternatives for synthetic larvicides. *Bacillus thuringiensis var. israelensis* (*Bti*) and *B. sphaericus* (*Bs*), gram-positive spore-forming bacteria species, are used as bacterial larvicides in many countries against *Anopheles* (Derua *et al*., 2019), *Aedes* (Boyce *et al*., 2013) and *Culex* (Dylo *et al*., 2014) mosquitoes. Although *Bs* has shown a better larval control in polluted water, their success in controlling *Aedes* larvae has been very poor (Rozendaal, 1997). The *Bti* products are available in the market as wettable powder and granules, consisted of dead bacteria, living spores and toxic crystals. Quick deposition of the material to the bottom has been identified as a drawback and slow-releasing briquettes and dunks, which float on the water, have been introduced to overcome this. Resistance development against bacterial larvicides caused by long-term application represents a serious threat to their success (Wirth, 2010).

Infective spores produced by entomopathogenic fungi such as *Lagenidium*, *Coelomomyces* and *Culicinomyces* can penetrate the mosquito cuticle and release toxins that kill the mosquito (Scholte *et al*., 2004). It has been shown that the rate of resistance development against fungal toxins is much slower than that against synthetic insecticides (Knols *et al*., 2010). It is anticipated that modern genetic engineering tools will assist in improving the fungal efficacy to control mosquito-borne disease vectors (Scholte *et al*., 2004).

Plant-based mosquitocides and green-fabricated nanoparticles

Another approach for mosquito control is employing bioactive attributes of plant-derived products (PDPs) which are of low mammalian toxicity and short environmental persistence. Azadirachtin (extracted from leaves and seeds of neem *Azadirachta indica*) and pyrethrum (extracted mainly from the flower *Chrysanthemum*) are the well-known PDPs used in mosquito control (George *et al*., 2014). Various plant extracts such as papaya (*Carica papaya*) leaf and seed extracts have shown mosquitocidal activities (Sesanti *et al*., 2014). In addition, a number of plant-based essential oils and extracts with mosquitocidal properties have been described (Shaalan *et al*., 2005). Herbal mosquito repellents are becoming popular due to their environmental friendliness, low cost and high effectiveness. Many researches have indicated that the natural plant extracted essential oils and other products exhibit better properties than synthetic ones (Kulkarni, 2017).

Today, the nano-biotechnology has revolutionized the field by synthesizing plant-mediated fabrications of nanoparticles to control mosquitoes (Benelli *et al*., 2016). Various green-synthesized metal nanoparticles (metal nanoparticles with bioactive agents such as plant extracts and microorganisms) have been made available. Silver-(protein-lipid) nanoparticles (Ag-PL NPs) fabricated using the seed extract of *Sterculia foetida* (Indian almond tree) have shown a very high efficacy against larvae of malaria vector *An. stephensi*, dengue vector *Ae. aegypti* and filariasis vector *C. quinquefasciatus* (Rajasekharreddy & Pathipati, 2014). *Sargassum muticum* (a seaweed) treated silver nanoparticles have reduced the egg hatchability of the same three mosquito species by 100 % (Madhiyazhagan *et al*., 2015).

RELEASING MOSQUITOES TO REDUCE TRANSMISSION OF MOSQUITO-BORNE DISEASES

Human population growth, increased globalization, rapid urbanization and geographic range expansion of mosquito species, together with increased vector resistance to insecticides and ineffective vector control programmes have led to the unprecedented global emergence of mosquito-borne diseases seen today. To effectively limit or prevent future outbreaks, intervention of novel strategies like releasing genetically modified mosquitoes or transgenic mosquitoes is essential. Two different endpoints are targeted by releasing mosquitoes: population reduction and replacement of vector population, with strains that are refractory to pathogen development and transmission (Figure 2).

Sterile insect technique (SIT)

The sterile insect technique (SIT) involves sterilization of male insects by radiation or chemicals to generate chromosomal aberrations/ dominant lethal mutations in sperms and release them in the wild. Any mating with released sterile males results in no progeny production leading to population suppression (Figure 2A). This strategy is associated with mass rearing of male insects and releasing them in very high proportion to wild population. Continuous release of sterile males will eventually eliminate the target insect population. The technique has been successfully implemented to eradicate the screwworm fly *Cochliomyia hominivorax* from North America to the Darien Gap in Panama (Wyss, 2000). Even though a SIT field trial carried out in Italian urban areas to control *Ae. albopictus* resulted in 70−80% sterility in the target population (Bellini *et al*., 2013), the technique is not widely recognized in mosquito control programmes. Accurate selection of male mosquitoes prior to release, irradiating large numbers of male mosquitoes, unavailability of a marker system to monitor the released mosquitoes, fitness disadvantage shown by irradiated males and continuous release have been identified as major drawbacks of SIT.

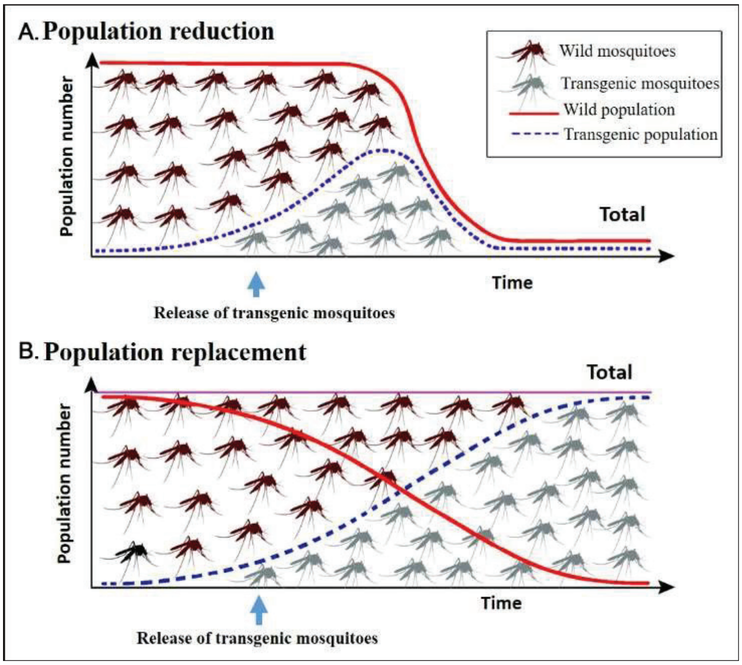


Figure 2: Graphical illustration of population reduction and population replacement by releasing transgenic mosquitoes.
A. Population reduction: conventional sterile insect technique (SIT), releasing mosquitoes carrying dominant lethal (RIDL) gene and *Wolbachia* mediated incompatible insect technique (IIT) are used to reduce a mosquito vector populations. B. Population replacement: release of transgenic mosquitoes and *Wolbachia* infected mosquitoes that are refractory to pathogen development and disease transmission can replace a mosquito population.

Releasing mosquito vectors carrying dominant lethal (RIDL) gene

Release of insects carrying a dominant lethal (RIDL) gene, a promising approach for reducing vector populations, was pioneered by the Oxitec (*www.oxitec.com*). In this system, transgenic insects carry a female-specific lethal dominant and repressible gene system (Thomas *et al*., 2000). Only males are released into the environment to mate with wild females so that the subsequent female progeny die in their immature stages or are flightless. The lethal gene is repressed using an antidote (tetracycline) so that female mosquitoes can be reared to adulthood in the laboratories in the presence of tetracycline for reproduction (Figure 3). This system also carries a fluorescence marker to monitor RIDL mosquitoes. A sustained series of field releases of OX513A *Ae. aegypti* males in a suburb of Brazil reduced the local *Ae. aegypti* population by 95% over a period of one year (Carvalho *et al*., 2015).

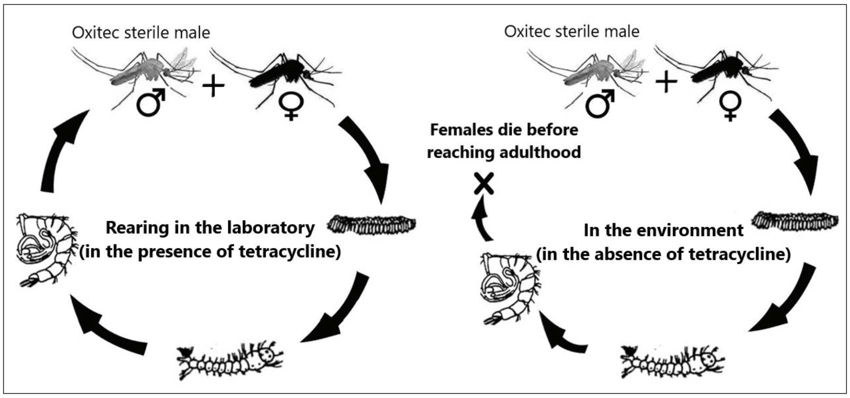
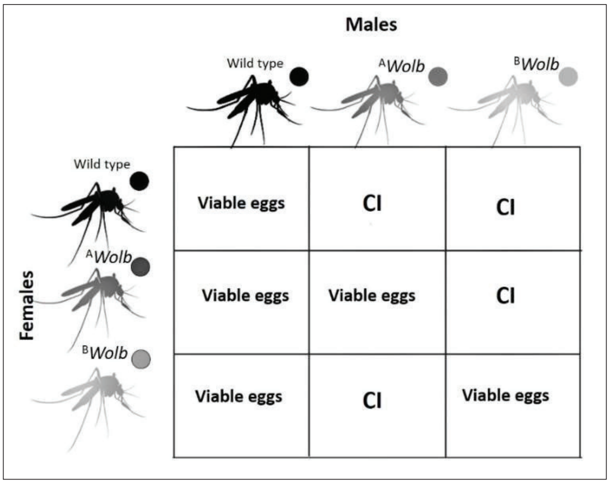


Figure 3: Oxitec release of insects carrying a dominant lethal (RIDL) gene method. Transgenic mosquitoes carry a female-specific lethal dominant gene which can be repressed using an antidote (tetracycline) so that female mosquitoes can be reared for reproduction in the laboratories in the presence of tetracycline.



Females \ Males	Wild type ●	^{A}Wolb ●	^{B}Wolb ●
Wild type ●	Viable eggs	CI	CI
^{A}Wolb ●	Viable eggs	Viable eggs	CI
^{B}Wolb ●	Viable eggs	CI	Viable eggs

Figure 4: *Wolbachia* induced cytoplasmic incompatibility (CI)

Releasing *Wolbachia* infected mosquitoes

The *Wolbachia*-based mosquito control has emerged as another novel and promising tool in mosquito control. *Wolbachia* are maternally inherited endosymbiotic bacteria that are naturally occurring in many arthropods including mosquitoes. *Wolbachia* infection causes reproductive abnormalities including feminization, male-killing and cytoplasmic incompatibility (CI) (Gotoh *et al*., 2006). Because of CI, viable offsprings are produced when *Wolbachia*-infected females mate with uninfected males or males infected with the same *Wolbachia* strain. If a *Wolbachia*-infected male mates with an uninfected female, or if a *Wolbachia*-infected female mates with a male infected with a different strain of *Wolbachia,* no offspring will be produced (Figure 4). The *Wolbachia*-induced CI was proposed as a potential tool for mosquito control as this can lead to a population reduction.

Various *Wolbachia* strains have been isolated from their natural hosts and transinfected to different mosquito vectors. It has been reported that different strains of *Wolbachia* in mosquitoes inhibit the transmission of pathogens such as dengue (DENV) (Hedges *et al*., 2008), chikungunya virus (CHIKV) (Aliota *et al*., 2016), yellow fever virus (YFV) (Van den Hurk *et al*., 2012), malaria parasites (Hughes *et al*., 2011) and Zika virus (ZIKV) (Dutra *et al*., 2016). Even though *Wolbachia* infection and associated disease control has been experimented with different vector mosquito species of the genera *Culex*, *Aedes* and *Anopheles* (Benelli *et al*., 2016; Yen & Failloux, 2020; Wang *et al*., 2021), the major focus has always been on *Ae. aegypti*, the primary vector of dengue. Transinfection of *Wolbachia* in *Ae. aegypti* is considered as a major success in dengue vector control since *Wolbachia* infection in *Ae. aegypti* is extremely rare in nature (Nugapola *et al*., 2017; Yen & Failloux, 2020).

Incompatible insect technique (IIT) uses infection of *Wolbachia* to prevent viable progeny production. When *Wolbachia*-infected males are released to a non-infected target population, a nonviable progeny will be produced leading to a population reduction. The IIT requires multiple release of mass number of *Wolbachia*- infected males. An IIT approach releasing of male *Wolbachia*-infected *Ae. aegypti* resulted in 92−98% reduction of the wild type population in Singapore (The Project *Wolbachia* – Singapore Consortium, 2021).

The IIT can be undermined by accidental release of females infected with the same *Wolbachia* strain. To overcome this, a combination of the radiation-based SIT and IIT can be advocated where female mosquitoes, if any, are exposed to radiation to ensure sterility (Lees *et al*., 2015) (Figure 5). Release of a triple infected *Ae. albopictus* (infected with three strains of *Wolbachia*) with a pupal irradiation to negate the effect of unintentional release of females, resulted in almost complete eradication of *Ae. albopictus* population in a field setting in China (Zheng *et al*., 2019).

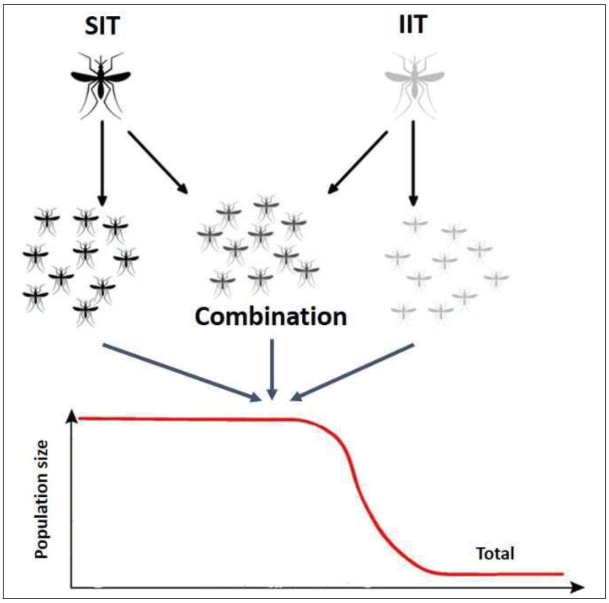


Figure 5: The three techniques, radiation-based sterile insect technique (SIT), incompatible insect technique (IIT) and the combined SIT/ IIT technique, to reduce vector populations

Wolbachia infection can also be used for vector population replacement strategy. Spreading *Wolbachia* into vector populations by releasing infected females can be regarded as a population replacement as shown in Figure 2B. Here, *Wolbachia* block the pathogen development and shorten the life of the mosquitoes reducing disease transmission. The population replacement strategy has been effectively used in Australia by infecting *Ae. aegypti* populations with the *w*Mel strain to reduce dengue transmission (Hoffmann *et al*., 2011). Infection with *w*MelPop resulted in almost complete blocking of transmission of DENV in field populations in Australia and Vietnam (Nguyen *et al*., 2015).

Since *Wolbachia* infection is maternally inherited, it can rapidly be spread across the population acting as a gene drive system (Segoli *et al*., 2014). Further, the *Wolbachia*-based gene drive system has its potential application to introduce foreign genes also to the symbiont- a process called paratransgenesis, to spread them into a vector population (Hayiril & Martelli, 2019). Release of mosquitoes to suppress or replace vector populations can be combined with auto-dissemination (Caputo *et al*., 2012) where released adults are used to disseminate substances such as IGRs. When IGR infested males are released, they will contaminate females and eventually IGR will be disseminated to breeding sites. This approach has been successfully implemented in Peru reducing *Ae. aegypti* populations (Devine *et al*., 2009).

Releasing transgenic mosquitoes with gene drive systems

Transgenic strains of mosquitoes have been developed with refractory genes (genes encoding anti-pathogen effector molecules) that reduce the mosquito vector competency. In order to effectively spread a refractory gene in a wild population, it can be coupled with a gene drive system which is capable of increasing the likelihood of the gene to be passed onto the next generation overriding normal inheritance.

Moving genes into a population is possible with gene drive systems developed through Maternal-effect dominant embryonic arrest (*Medea*) (Chen *et al*., 2007) and Clustered Regularly Interspaced Palindromic Repeats (CRISPR)/Cas9 systems (Doudna & Charpentier, 2014). CRISPR/Cas9 system is a highly effective tool for precision genome-editing (Kistler *et al*., 2015) and can be used for population suppression and population replacement. This can be used to modify a vector population to express anti-pathogenic effector molecules as a population replacement strategy (Adolfi *et al*., 2020). Current gene-drives also employ a CRISPR-based DNA cleavage mechanism to copy themselves from their location on one chromosome to the same site on another chromosome. This can alter the balance of naturally occurring genetic variants and is referred to as allelic-drive. Inclusion of additional CRISPR components to cut the unfavoured allele but not the preferred allele can favour the inheritance of one allele over another. When this process occurs in germ cells the unfavoured allele is corrected by copying the gene sequence of the intact preferred allele. A fascinating CRISPR gene-drive system developed in mosquitoes had the ability to be transmitted in a highly efficient "super-Mendelian" fashion to >99% of progeny and carried a gene cassette conferring resistance to malarial parasites (Grantz *et al*., 2015). This allelic-drive process has been used to replace an allele conferring resistance to DDT with the native insecticide susceptible allele in the fruit fly *Drosophila melanogaster* indicating the potential of restoration of insecticide susceptibility in mosquito populations reversing insecticide resistance (Kaduskar *et al*., 2022).

CONCLUSIONS AND FUTURE PERSPECTIVES

Various strategies and techniques have been used to control mosquitoes aiming to reduce transmission of mosquito borne diseases. Each method has its own merits and demerits. Even though the control programmes still heavily depend on conventional insecticides, an urgent need has been arisen to limit their usage mainly due to resistance development and environmental concerns. Biological control strategies including the use of natural enemies and plant- and microorganism- based bioactive compounds are eco-friendly but often have operational difficulties in large-scale use, and are hence recommended for integrated vector control approaches. Two novel technologies, RIDL and *Wolbachia* mediated mosquito control, have given promising results in controlling wild mosquito populations reducing disease transmission. Advancement in gene drive systems will allow us to effectively spread refractory genes and insecticide susceptible alleles into mosquito populations overriding normal inheritance to aid in mosquito borne disease control. Enrichment of our knowledge on mosquito biology and the changing behaviour of mosquito species are of paramount importance in developing new molecular tools for effective mosquito control. Technological advances provide novel approaches to solve old problems. Such is the case with mosquito control!

Conflict of interest

The authors declare that there is no conflict of interest.

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RESEARCH ARTICLE

Ocean circulation around Sri Lanka

CB Pattiaratchi[1*], EMS Wijeratne[1] and A de Vos[1,2]
[1] *Oceans Graduate School & The UWA Oceans Institute, The University of Western Australia, 35 Stirling Highway, Crawley, Western Australia 6009, Australia.*
[2] *Oceanswell, Oceanswell, 9 Park Gardens, Colombo 5, Sri Lanka.*

Abstract: Sri Lanka, located in the northern Indian Ocean with the Arabian Sea on its western side and the Bay of Bengal on its eastern side and experiences bi-annually reversing monsoon winds. This brief overview explored the dynamics of the surface circulation and coastal upwelling in the waters around Sri Lanka, particularly along the southern coast, using satellite imagery and numerical simulations using the Regional Ocean Modelling System (ROMS). The results confirmed the presence of the reversing current system, between the equator and Sri Lanka, in response to the changing wind field: the eastward flowing Southwest Monsoon Current (SMC) during the Southwest (SW) monsoon and the westward flowing Northeast Monsoon Current (NMC) during the Northeast (NE) monsoon, respectively. Along the eastern and western coasts, during both monsoon periods, flow is southward converging along the south coast. During the SW monsoon the Island deflects the eastward flowing SMC southward whilst along the east coast the southward flow results from the Sri Lanka Dome recirculation. The major upwelling region was located along the south coast resulting from southward flow converging along the south coast and subsequent divergence associated with the offshore transport of water. The location of the flow convergence and hence the upwelling centre was dependent on the relative strengths of wind driven flow along the east and west coasts: during the SW (NE) monsoon the flow along the western (eastern) coast was stronger migrating the upwelling centre to the east (west).

Keywords: Island-mass effect, monsoon, Sri Lanka, surface circulation, upwelling.

INTRODUCTION

The Island of Sri Lanka occupies a unique geographic/topographic and oceanographic location in the equatorial northern Indian Ocean, with the Arabian Sea on its western side and the Bay of Bengal on its eastern side (Figure 1). The Island is an extension of the Indian sub-continental landmass into the ocean with deeper water (water depths > 2000 m) on either side of the Island. The interaction between the ocean currents and the Island topography controls the ocean circulation (de Vos *et al*., 2014). From an oceanographic perspective, location of Sri Lanka is unique with its offshore waters transporting water with different properties, originating from Bay of Bengal and the Arabian Sea, through reversing ocean currents, driven by monsoon winds (Schott & McCreary, 2001; Su *et al*., 2021). The northern Indian Ocean is characterised by bi-annually reversing monsoon winds resulting from the seasonal differential heating and cooling of the continental land mass and the ocean. The Southwest (SW) monsoon generally operates between June and October and the Northeast (NE) monsoon operates between December through April (Tomczak & Godfrey, 2003). The transition periods are termed the First Inter-Monsoon (May) and Second Inter-Monsoon (November). During the SW monsoon, the Southwest Monsoon Current (SMC) flows from west to east transporting higher salinity water from the Arabian Sea (Figure 2a) whilst during the NE monsoon the currents reverse in direction with the Northeast Monsoon Current (NMC) transporting lower salinity water originating from the Bay of Bengal from east to west (Figure 2b). During the SW monsoon, increased chlorophyll concentrations (> 5 mg m^{-3}) have been recorded around Sri Lanka, particularly along the southern coast (Vinayachandran *et al*., 2004; de Vos *et al*., 2014) which appears to be a major upwelling region.

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[*] Corresponding author (chari.pattiaratchi@uwa.edu.au; https://orcid.org/0000-0003-2229-6183)

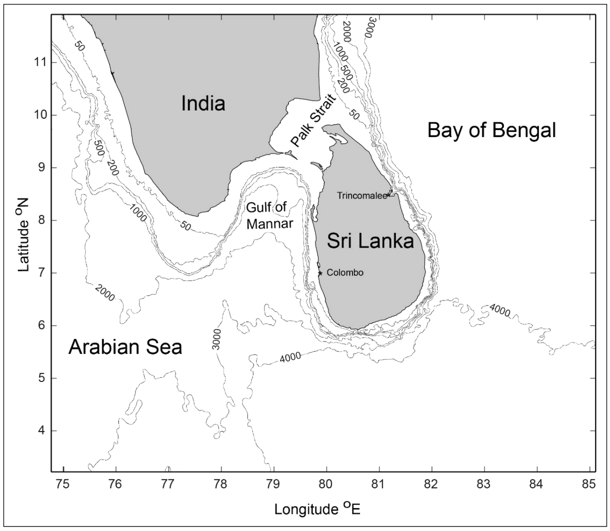

Figure 1: Study area showing bathymetry and model domain

The continental shelf around Sri Lanka is narrower, shallower and steeper than is average for the world (Wijeyananda, 1997). Its mean width is 20 km, and it is narrowest on the southwest coast where it is less than 10 km (Shepard, 1963; Swan, 1983; Wijeyananda, 1997). The continental slope around Sri Lanka is a concave feature that extends from 100 m to 4,000 m in depth. The continental slope on the southern and eastern coasts has an inclination of 45° which is one of the steepest recorded globally (Sahini, 1982). The abyssal plain around the island is 3,000–4,000 m deep (Swan, 1983).

The seasonal difference of sea surface salinity (> 2 ppt) around Sri Lanka is highly significant compared to other regions (Levitus *et al*., 1994). Salinity in the Bay of Bengal is generally lower (< 33 ppt), whilst salinities in the Arabian Sea are higher with maxima up to 36.5 ppt due to high evaporation and negligible freshwater input. The exchange of these waters occur along the southern coast of Sri Lanka with the reversing currents due to the monsoon winds.

There is significant seasonal variation in sea level in the north-eastern Indian Ocean with a range in the inner Bay of Bengal of ~0.80–0.90 m decreasing to the south (Wijeratne, 2008), hence mean sea level is 0.05 m lower in January compared to July, due to the inverse barometric effect. The seasonal sea level variability around Sri Lankan waters is around 0.2-0.3 m with maxima during June through the action of the SW monsoon (Wijeratne *et al*., 2008). The tides around the Island are mixed semidiurnal with a maximum spring tidal range of ~0.70 m.

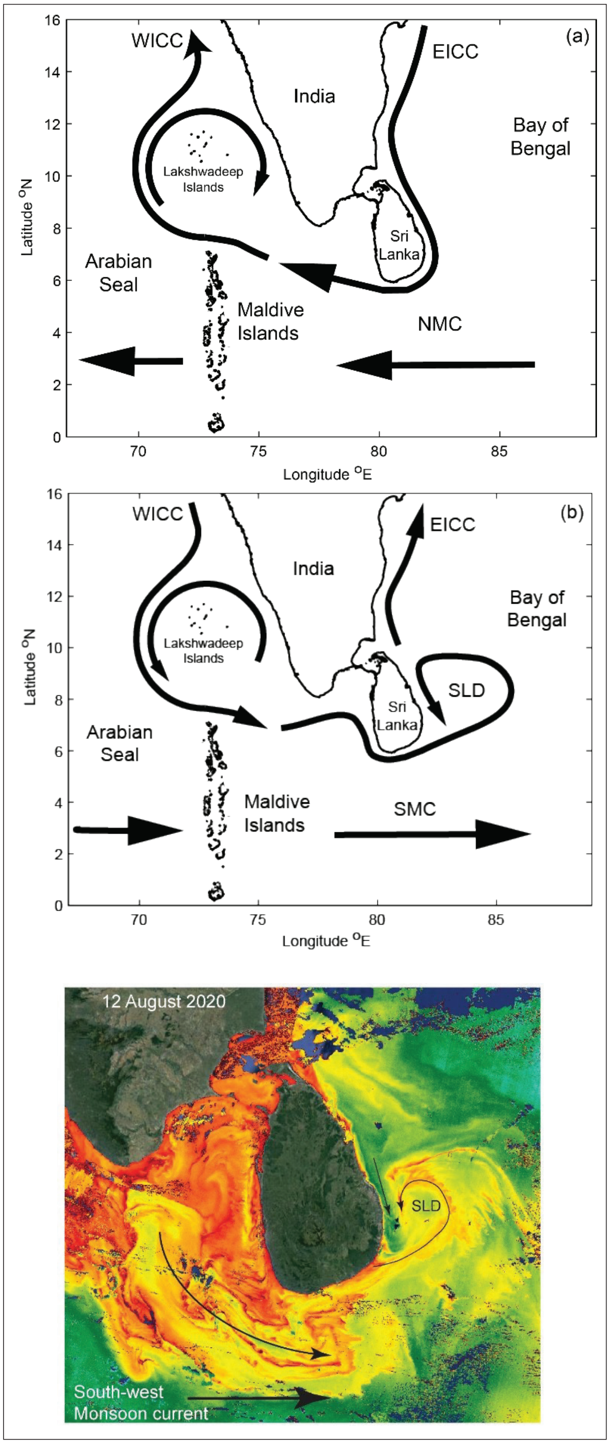

Figure 2: Circulation patterns around Sri Lanka and southern India for (a) Northeast monsoon; (b) Southwest monsoon; (c) ocean colour image from 12 August 2020 showing surface chlorophyll concentration. WICC – West Indian Coastal Current; EICC – East Indian Coastal Current; SMC – South Monsoon Current; NMC – North Monsoon Current; SLD– Sri Lanka Dome.

A schematic of the circulation in the northern Indian Ocean, in the vicinity of Sri Lanka is shown in Figure 2 (Schott & McCreary, 2001; de Vos *et al*., 2014; Su *et al*., 2021). During the SW monsoon along India and Sri Lanka (Figure 2b), the eastern boundary current or West Indian Coastal Current (WICC) in the Arabian Sea flows southwards along the West Indian coastline and joins the eastward flowing Southwest Monsoon Current (SMC). The presence of the anti-clockwise Lakshadweep eddy off the southwest coast of India modifies the current flow in this region. The SMC flows along the south coast of Sri Lanka from west to east transporting ~11.5 Sv (1 Sv = 10^6 m^3s^{-1}) between the equator and Sri Lanka (de Vos *et al*., 2014). After passing the coast of Sri Lanka, the currents form an anti-clockwise eddy defined as the Sri Lanka Dome (SLD) centred around 83° E and 7° N (Vinayachandran & Yamagata, 1998; de Vos *et al*., 2014; Su *et al*., 2021). The western arm of this eddy drives a southward current along the east coast of Sri Lanka (Figure 2c) whilst the remainder flows northward along the eastern Indian coast as the East Indian Coastal Current (EICC).

During the NE monsoon the currents reverse in direction (Figure 2a). Along the eastern Indian coast, the EICC flows southward past Sri Lanka and joins the Northeast Monsoon Current (NMC) flowing from east to west transporting ~9.6 Sv (de Vos *et al*., 2014). The currents then flow around the clockwise Lakshadweep eddy and northward along the western Indian coastline as the West Indian Coastal Current (WICC). One of main features to note from this description from the perspective of Sri Lanka, is the reversal of currents along the western and southern coasts and the north to south flow along the eastern coast.

Sri Lanka is a relatively large island (length 440 km; width 225 km), extending offshore into the Indian Ocean, similar to a headland. This allows for the Island to interact with the seasonally reversing monsoon. Many studies have reported the influence of flow interaction with islands and headlands leading to enhanced primary production – termed the island mass effect (IME) by Doty and Oguri (1956). These studies have included different spatial scales using laboratory and field experiments to understand circulation and enhanced productivity (Su *et al*., 2021).

The upwelling off the south coast of Sri Lanka usually appears and intensifies during the summer months when the SW monsoon prevails. Monthly satellite image composites of chlorophyll analysed by Yapa (2009) show high productivity waters with mean chlorophyll concentrations > 5 mg m^{-3} along the southern and western regions during the months of June-August that are accompanied by a 2° to 3° C decrease in sea surface temperature (SST) corresponding to regions where high chlorophyll concentrations are detected.

This paper is brief review of the circulation around Sri Lanka with an emphasis on the seasonal changes in circulation and upwelling patterns along the southern coast of Sri Lanka using satellite imagery and a high-resolution numerical model (ROMS).

METHODOLOGY

The main approach for the study is the use of satellite imagery and a numerical model to describe the mean circulation patterns and upwelling around Sri Lanka. Meteorological information from ECMWF ERA interim data which were also used for model forcing have been used to define the seasonal wind climate.

ROMS configuration and validation

The Regional Ocean Modelling System (ROMS) is a three-dimensional numerical ocean model based on the nonlinear terrain following coordinate system of Song and Haidvogel (1994). ROMS solves the incompressible, hydrostatic, primitive equations with a free sea surface, horizontal curvilinear coordinates, and a generalized terrain-following s-vertical coordinate that can be configured to enhance resolution at the sea surface or seafloor (Haidvogel *et al*., 2008). This model set-up is an extension of the same configuration used in de Vos *et al*. (2014) and Pattiaratchi *et al*. (2022).

The model grid (Figure 1) configured for this study included the continental shelf and slope waters surrounding Sri Lanka as well as the deeper ocean and consisted of a horizontal grid with resolution < 2 km with 30 vertical layers in a terrain-following s-coordinate system. The model was driven by direct air-sea heat and freshwater fluxes, momentum fluxes, inverted barometric effect, tide/sea level, transport and tracers at open boundaries. The forcing data were interpolated onto the corresponding model grid points to create initial and forcing files. The model was driven with 3 hourly atmospheric forcing and daily surface heat and freshwater fluxes using ECMWF ERA interim data. The heat and freshwater fluxes were also specified using ECMWF ERA data. The net heat flux at air-sea interface was estimated based on the balance of incoming solar radiation, outgoing long wave, sensible and latent heat fluxes, respectively. Freshwater fluxes were estimated using precipitation and evaporation data from ECMWF ERA data and the river inputs were ignored.

Satellite imagery

Suspended material (such as sediment, chlorophyll etc) in the surface waters may be used as a passive tracer to follow flow patterns using satellite imagery (Pattiaratchi *et al*., 1987). In regions of upwelling (for example see Figure 3), there is also a correspondence between regions of higher surface chlorophyll concentrations (SCC) and lower sea surface temperatures (SST). Thus, ocean colour imagery may be used to qualitatively validate numerical model outputs. Comparison between predicted SST and satellite derived SCC indicate that the model reproduced observed patterns, particularly the higher chlorophyll 'tongue' feature, and sharp fronts. Satellite imagery acquired by the Sentinel-3 satellite was used to supplement the ROMS model output. Here, the surface chlorophyll concentrations estimated using the OLCI algorithm was used (*https://ovl.oceandatalab.com*).

RESULTS

The wind field

The monsoon and inter-monsoon periods occur at similar times during the year. However, there is an inter-annual variability in the onset of these climatic events and thus the timing of each monsoon can vary by up to 1-2 months. In addition to the seasonal changes in the wind field there is also significant spatial distribution as revealed by the ECMWF ERA interim data (Figure 3). One of the factors influencing the spatial wind field is the local land topography of Sri Lanka and southern India. Coastal regions around Sri Lanka are relatively flat and surround the elevated central region that increases to a maximum elevation of 2,500 m. Similarly, southern India consists of elevated terrain that exceeds 1,000 m (Luis & Kawamura,

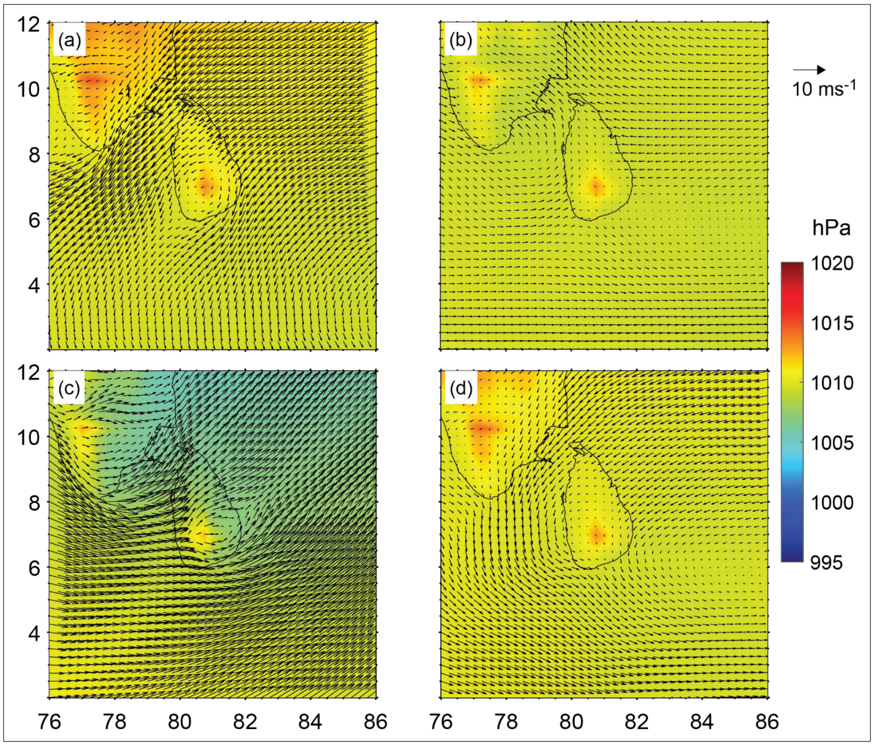

Figure 3: Seasonal mean wind speeds and directions for 2021 from ECMWF ERA interim data. Each plot represents a bi-monthly average with the length of arrow correlating with speed. (a) January-February (northeast monsoon); (b) April-May (first inter-monsoon); (c) July-August (southwest monsoon); (d) October-November (second inter-monsoon). The higher surface atmospheric pressure reflects the higher topography in the center of Sri Lanka and southern India.

2000). These are highlighted by the relatively higher surface atmospheric pressure at the centre of Sri Lanka and southern India (Figure 3). During the NE monsoon (Figure 3a), winds are predominantly from the northeast across the study region with stronger winds in the Gulf Mannar (Figure 1). Here, the north-easterly winds are funnelled through the elevated topography between southern India and Sri Lanka resulting in strong winds over the Gulf of Mannar (Luis & Kawamura, 2000). Off the southern coast of Sri Lanka, the winds are weaker and are mainly offshore during the NE monsoon (Figure 3a). The interaction between the NE monsoon winds and the central highlands of Sri Lanka results in a region of lower wind speeds ('wind shadow') off the south-west coast (Figure 3a). During the first inter-monsoon, the east coast of Sri Lanka experiences onshore winds (easterly) with weaker winds off the south coast remaining offshore (Figure 3b). Along the western and southern coasts of Sri Lanka, during the SW monsoon, the winds are stronger and are westerly (Figure 3c) and, due local topography, they veer northwards off the eastern side of the island (south-westerly winds). The influence of the central highlands is more pronounced with the wind shadow extending significantly offshore (Figure 3c). During the second inter-monsoon, the east coast of Sri Lanka experiences weaker north-easterly winds with winds off the southern coast remaining offshore and northerly (shore parallel) along the west coast (Figure 3d).

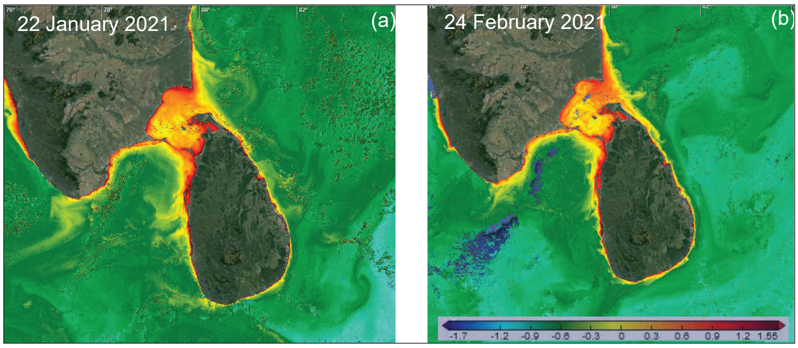

Figure 4: Weekly mean surface chlorophyll concentration (SCC) from Sentinel-3 satellite during the north-east monsoon in 2021: (a) 22 January 2021; and, (b) 24 February 2021. The SCC scale in (b) is logarithmic and has units mgm^{-3}.

Seasonal circulation

Satellite imagery

The seasonal circulation around Sri Lanka was examined through the use of surface chlorophyll concentration (SCC) for north-west (2021) and southwest monsoon periods. The SSC is used as a passive tracer to follow flow patterns using satellite imagery.

In January, the Northeast Monsoon Current (NMC) flows from east to west (Figure 2a) with offshore winds along the west coast (Figure 3a). In general, the weekly mean SCC was low around the Island and relatively higher values were restricted to the narrow coastal region (Figure 4). This is reflected in the SCC data with slightly higher concentrations to the west of Sri Lanka. There is evidence of offshore advection of SCC along the west coast.

In May, during the onset of the SW monsoon (Figure 2b) the flow is mainly from west to east along the south coast and a band of higher SCC (~2.0 mgm^{-3}) water was present along the south coast of Sri Lanka and is advected to the east (Figure 5a). The low SCC concentrations along the east coast of Sri Lanka was due to the southward flow of water (see Figure 2b, c). In the time series of images between 7 May and 26 June 2019, we follow the development of a very narrow band of higher SCC advecting to the east (Figure 5). This band is formed through the convergence of the eastward flowing water advecting higher SCC water from the south coast to the east and the southward flowing lower SCC water along the east coast (see also Figure 2c). On 14 May the only a small feature may be seen along the south coast (Figure 5b) and over the following weeks (to 10 June) the narrow band is a consistent feature (Figures 5c–e). On 26 June the band has contracted to the west and is now wider than the previous weeks (Figure 5f).

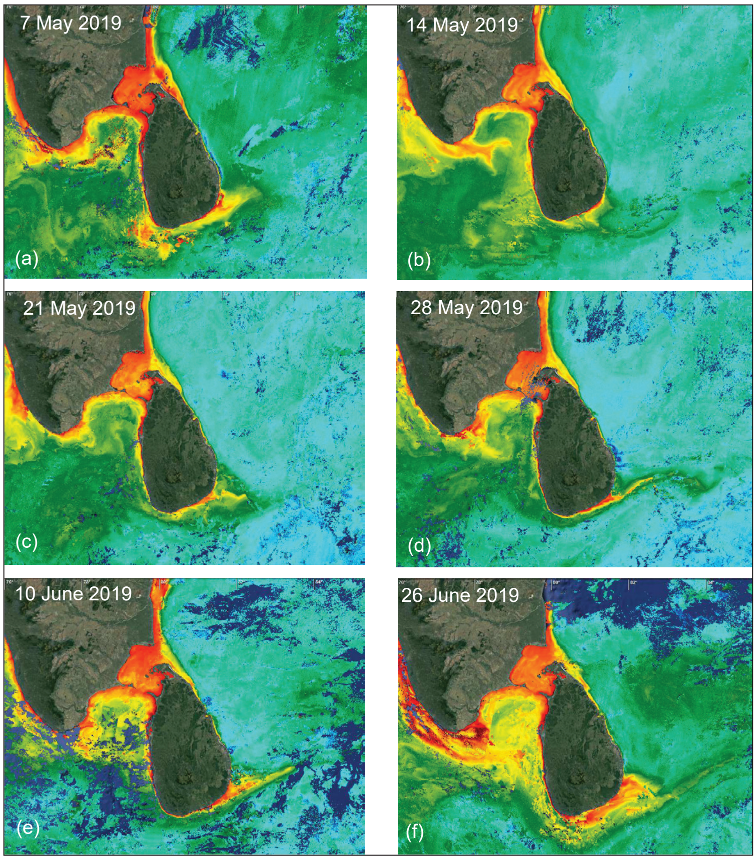

Figure 5: Weekly mean surface chlorophyll concentration from Sentinel-3 satellite during the onset of the south-west monsoon in 2019: (a) 7 May 2019; (b) 14 May 2019; (c) 21 May 2019; (d) 28 May 2019; (e) 10 June 2019; and, (f) 26 June 2019. Colour scale of SCC is shown on Figure 4b.

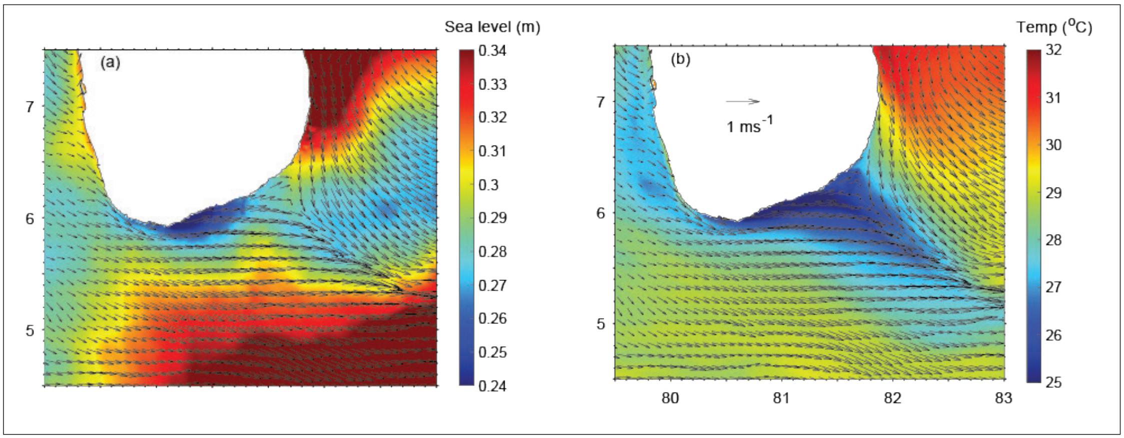

Figure 6: Predicted near-surface current vectors superimposed (a) sea level; and, (b) sea surface temperature along the southern coast during the south-west monsoon

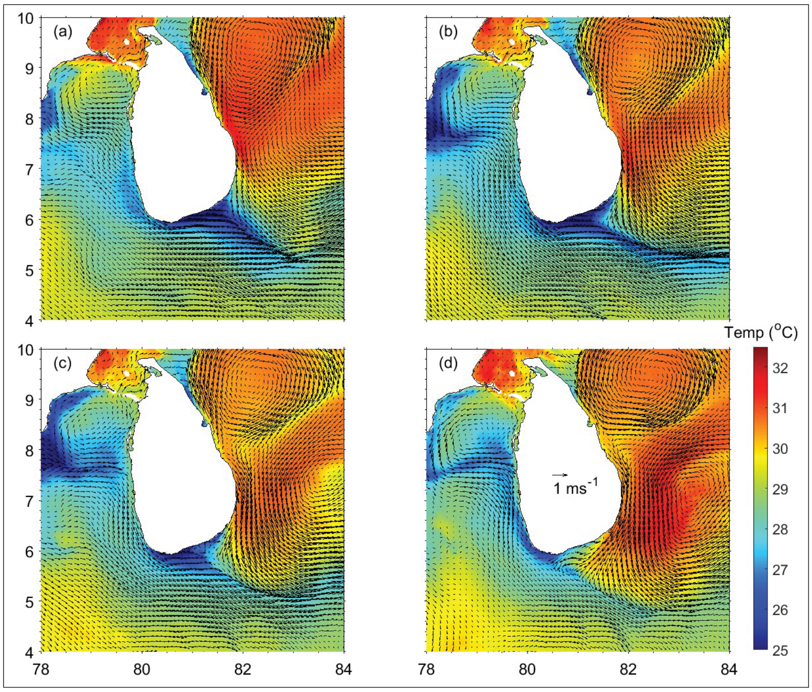

Figure 7: Predicted near-surface current vectors with sea surface temperature around Sri Lanka during south-west monsoon. (a) 11 June 2021; (b) 14 June 2021; (c) 17 June 2021; and, (d) 20 June 2021.

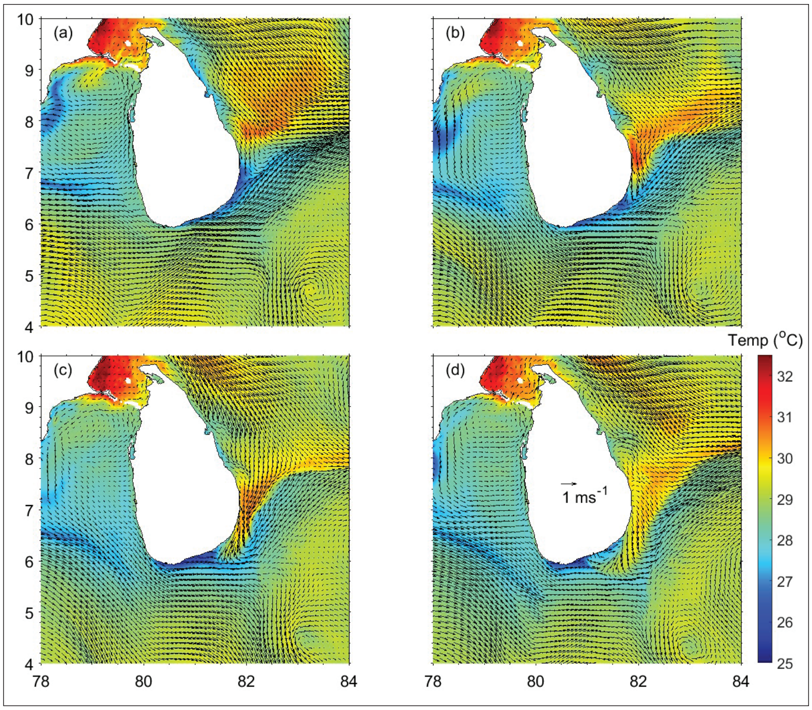

Figure 8: Predicted near-surface current vectors with sea surface temperature around Sri Lanka during late north-east monsoon and first inter-monsoon. (a) 09 Oct 2021; (b) 11 Oct 2021; (c) 13 Oct 2021; and, (d) 15 Oct 2021

Numerical modelling

Numerical model results provide additional information on the general patterns identified in previous studies (Figure 2) and ocean colour imagery (Figure 5). These were fully described in de Vos *et al*. (2014) and for the larger scale by Su *et al*. (2021). The numerical model results presented here are limited to flow features along the southern coast of the Island during the south-west monsoon: the formation of a cold-water tongue extending from the coast to offshore that was also present identified in the ocean colour imagery (Figure 5). We define the 'cold water tongue' as the cold-water patch that begins at the coast and extends offshore, narrowing with distance similar to a triangle (e.g. Figure 6b). This cold-water tongue is present due to the convergence of two current streams (Figure 2b, c): (1) eastward flow along the south coast as part of the south-west monsoon current; and, (2) the southward flow along the east coast of Sri Lanka as part of the Sri Lankan dome. As part of this convergence feature the mean sea level is depressed along the south coast with higher sea levels along the east coast and offshore (Figure 6a). The decreased sea level at the coast results in upwelling of colder water that is advected offshore by the convergent currents (Figure 6b). The colder water originates from water depths of 80–100 m (Figure 11; Su *et al*., 2021).

The ocean is dynamic and changes at time scales of order days reflect changes in atmospheric forcing. De Vos *et al*. (2014) identified changes occurring over 10-day cycles. The model predicted daily mean sea surface temperature (SST) and flow fields were examined at shorter timescales over a 10-day period during the south-west monsoon period (Figures 7 and 8). From 11–14 June 2021 (south-west monsoon) – the cold-water tongue was extending in a south-east direction and the convergence of the currents along the south coast (from the west) and east coast (from the north) can be readily identified (Figure 7a, b). On 17 June, the currents along the east coast has strengthened mainly due to the formation of an anti-clockwise eddy centred at 83.5°E 7.5°N and the cold water tongue has moved slightly to the west (Figure 7c). By 20 June, the eddy has strengthened further, and stronger southward currents are present along the east coast of the Island and this has resulted in the cold water tongue being moved further to the west and becoming weaker in terms of the colder water (Figure 7d). On 9 October, due to the stronger currents along the southern coast, the cold-water tongue with converging currents evident is now present further to the east, along the southern section of the east coast (Figure 8a). Over the period 11–15 October, the currents along the east coast have strengthened and the cold water tongue shifted to the east (Figures 8b–d). These daily model outputs confirm the observations through ocean colour imagery (e.g. Figure 5) and those reported by de Vos *et al*., 2014).

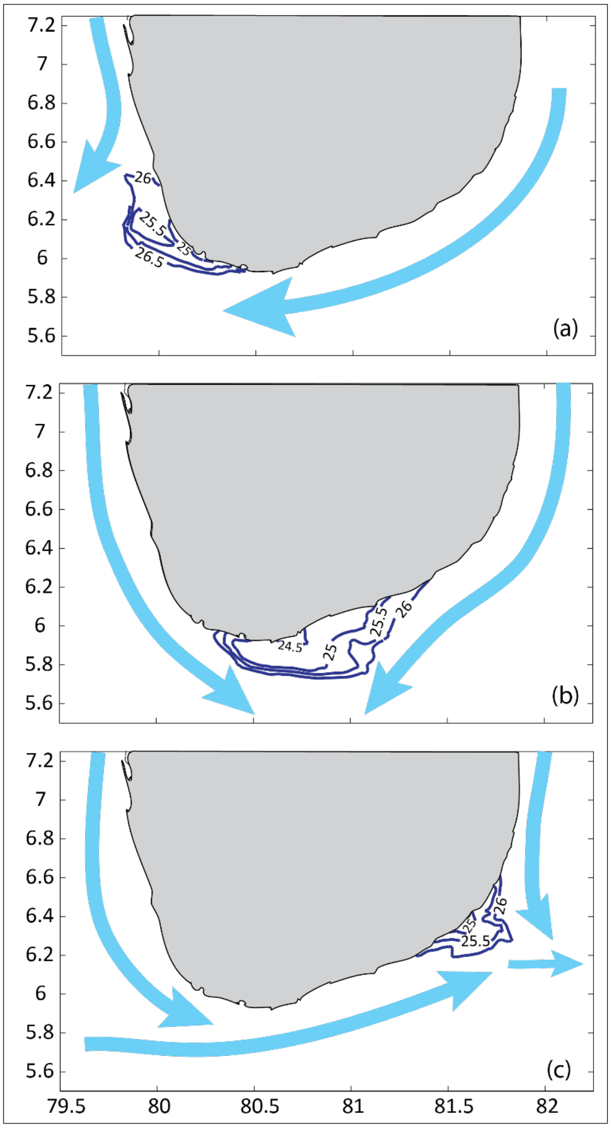

Figure 9: Predicted locations of the convergence region and associated upwelling region with respect to different current strengths along each coast (modified from de Vos *et al*., 2014).

The model results and ocean colour imagery indicated that the upwelling and associated colder, higher chlorophyll water along the southern coast of Sri Lanka during the south-west monsoon was due to flow convergence from currents flowing in different directions along the coast: to east along the south coast and to the south along the east coast. It was not due to direct wind driven upwelling through Ekman dynamics. Location of the upwelling centre depends in the relative strength between the two current systems and is shown schematically in Figure 9. When the currents from the west are stronger, the centre of cold-water tongue is located in the south-east (Figure 9a; see also Figure 3; de Vos *et al*., 2014). When the current strengths are comparable, the upwelling centre is located along the central part of southern coast (Figure 9b) and when the currents from the east are stronger, the centre of cold-water tongue is located in the south-west (Figure 9c).

DISCUSSION AND CONCLUSIONS

The seasonal and shorter term (< 10 days) changes in the surface circulation and upwelling patterns around Sri Lanka were examined using satellite imagery (mainly ocean colour) and a high spatial resolution numerical model (ROMS) configured to the study region and forced with ECMWF interim data. The emphasis was on the southern coast of Sri Lanka. The model reproduced all of the documented major circulation features in the region (de Vos *et al*., 2014; Su *et al*., 2021): reversing monsoon currents in response to the changing wind field and the Sri Lanka Dome. During both the SW and NE monsoons, the model results indicated southward flow along both east and west coasts converging and then moving offshore along the south coast. As the water is flowing offshore, there is divergence of water at the coast which results in upwelling of colder water from depth. This was confirmed by the numerical model output which indicated a lower sea surface height at the centre of upwelling.

Knowledge of ocean circulation and upwelling patterns are critical for managing anthropogenic impacts in the oceans. Upwelling regions are high primary production regions due to supply of nutrients from the sub-surface and therefore are important for the fishing industry. In many regions globally, the productive fishery grounds are located along ocean fronts that separate colder and warmer waters. Knowledge of these regions and how they move are critical for the fishing industry and for the prediction of potential fishing zones (e.g. *http://www.nara.ac.lk/?page_id=14683*). The surface ocean circulation is critical for the prediction of dispersion of buoyant material such as oil and plastics. These were highlighted by recent maritime accidents in Sri Lankan waters associated with the MT *New Diamond* and MV *X-Press Pearl* incidents.

MT *New Diamond*, was a large crude oil carrier transporting approximately two million barrels (~270,000 tonnes) of crude oil from Mina al Ahmadi in Kuwait to Paradip refinery along the east coast of India. On 3 September 2020, a fire broke out in the engine room of the ship. At this time, the ship was ~65 kilometres off Sangaman Kanda Point along the east coast of Sri Lanka. After burning intermittently for almost a week, the fire was finally extinguished on 11 September 2020. However, a diesel oil spill that was ~1 km long was observed during this period. During this time the winds and currents were directed offshore and thus there was no impact at the coast. Interestingly, when the accident occurred the ship was located close to the convergence region of the cold-water tongue (Pattiaratchi & Wijeratne, 2020).

The container ship, MV *X-Press Pearl*, was transporting cargo from Jebel Ali (United Arab Emirates) to Colombo (Sri Lanka) via Hamad Port (Qatar) and Hazira (India). The ship departed the port of Hazira on 15 May 2021 and arrived in Colombo on 19 May and was anchored off Colombo Port ~9.5 km offshore in a water depth of 21 m. On 22 May, a fire started in the cargo hold and by 24 May fire had intensified burned for 13 days. On 2 June, efforts to move the ship into deeper waters failed, with the aft portion sinking to the seabed. The vessel was transporting 1,486 containers, with a mixed variety of cargo but included 78 tonnes of plastic nurdles (low-density polyethylene pellets) in three containers that were released to the ocean (Pattiaratchi & Wijeratne, 2021). Nurdles are small plastic pellets used as raw materials for the manufacture of virtually anything that is plastic (e.g., from plastic bags and bottles to automobile parts) and are by definition classified as microplastics as their size is < 5mm (Pattiaratchi *et al*., 2022). During the period of the incident, there were strong onshore winds (> 10 ms^{-1}) and swell waves (height ~2 m) and was associated with the beginning of the south-west monsoon with the currents flowing to the south along the west coast of Sri Lanka at the location of the accident and to the east along the south coast. The numerical model simulations (using the same model as described in this paper) indicated movement of the nurdle plume southwards with the prevailing currents and by 27 May the plume had extended to the south-west corner of the Island. On 29–31 May, the nurdle plume had detached from the coast and was moving offshore to the south-east (Pattiaratchi *et al*., 2022).

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REVIEW

Nanotechnology in sustainable value addition to Sri Lankan minerals

RMG Rajapakse*
Department of Chemistry, Faculty of Science, University of Peradeniya, Peradeniya, Sri Lanka.

Summary: Sri Lanka is an Island with high quality natural resources such as precious minerals, endemic flora, and fauna, abundantly available throughout its land and sea. However, the country is among those with the poorest national economies in the world. The economy of the country is progressively declining due to the lack of a focused programme which would direct the country towards an improved economy. We, as academics and researchers, have devoted our time and energy towards developing value-added products from local minerals. This review describes some important natural minerals of Sri Lanka and our research activities on adding value to these natural treasures. The review begins with discussing natural resources of Sri Lanka focusing on industrial minerals. It then reveals the importance of adding value to these natural resources. Quoting our own research publications, the review describes the methodologies and benefits of adding value to Sri Lankan minerals. The review emphasizes the importance of preventing the export of valuable minerals in their raw forms by analysing market research on value added minerals.

Keywords: Apatite, feldspar, graphite, ilmenite, natural resources, quartz.

NATURAL RESOURCES OF SRI LANKA

Sri Lanka is gifted with several Natural Resources which include very high-quality natural minerals and endemic flora and fauna. Sri Lankan earth accommodates precious gemstones, pearls, very high-quality graphite, 100% pure quartz, mineral sands containing ilmenite, rutile and monazite, massive deposits of calcite, dolomite, apatite and iron phosphates and valuable clay minerals and so on. Some of these minerals are shown in Figure 1.

Sri Lankan graphite

Sri Lanka is one of five countries where high purity natural vein graphite is abundantly available. The other four countries where vein graphite localities available are Great Britain (Borrowdale and Cumberland), USA (North-western New Jersey, Dillion in Montana, and Ticonderoga), India (Kerala and Korani), and Madagascar (Ambohijanaka village in the Andramasina region). The vein graphite is known as crystalline vein, plumbago, Sri Lankan graphite, and Ceylon graphite; the latter names are due to the fact that Sri Lanka is the only country in the world where vein graphite is commercially produced in large quantities for the export market. This has occurred since the Dutch colonization era and peaked during the period of the second World War. There are reports to the effect that unusual graphite deposits of Ceylon have been known, and apparently used locally, since the middle of the 1600s. The fact that Sri Lanka is exporting its valuable highest quality natural vein graphite without any value addition should not be encouraged. Although, recently, Sri Lankan graphite mines are considering adding value to graphite by developing various products and making graphite aqueous dispersions, expanded graphite and graphene products, most of such work is done elsewhere in other countries such as Germany and Australia because some of the famous Sri Lankan graphite mines have been sold to companies from these countries. Therefore, their contribution to the national economy is minimal. The graphite market has escalated recently due to their use in batteries for electrical cars and a new market avenue is

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* rmgr1521961@gmail.com; https://orcid.org/0000-0003-3943-5362

opening at the Tesla company. We have developed novel processes to convert graphite to expanded graphite and graphene products. This will be discussed in detail in a latter section.

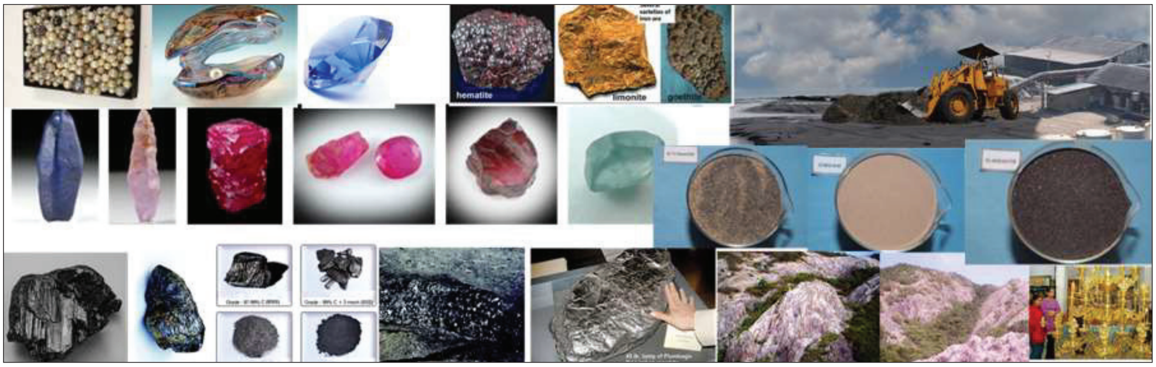


Figure 1: Some of the minerals naturally found in Sri Lanka. Photographs were taken from websites of respective industries.

Sri Lankan quartz

Sri Lanka is also gifted with high purity quartz containing over 98% pure silica. Such vein quartz deposits of high purity (over 98% silica) are found in Galaha (Kandy), Rattota, Balangoda, Pelmadulla, Embilipitiya and Ratnapura. Quartz has many technological applications including filters and absorbents, foundry sand, fillers, abrasives, specialized glassware used in laboratories, cuvettes for UV-visible spectroscopy, high tech products of ultrapure quartz for optical fibres, and for solar grade silicon manufactured for use in the electronics industry and photovoltaic cells. The silicon production increased from 35 ktonnes in 2005 to 105 ktonne in 2010. Although Sri Lankan scientists have already developed technologies to produce industry-specific quartz raw material using Sri Lankan vein quartz, these technologies are yet to be incorporated on a commercial scale (Pathirage *et al*., 2019; Udawatte *et al*., 2020). The conversion of quartz into solar grade silicon involves heating with coke at high temperatures. For each tonne of silica converted to silicon, ten tonnes of carbon dioxide are emitted to the atmosphere. We are developing an electrochemical process that does not require coke for the conversion of silica in quartz to silicon. This will eliminate undesirable environmental consequences due to excessive carbon dioxide emission. This will be discussed in detail later.

Sri Lankan ilmenite

Ilmenite is a commonly found in Sri Lankan mineral sands particularly in the Pulmudai area. Ilmenite is composed of iron titanate $FeTiO_3$ and is a major source used to extract titanium dioxide particles and titanium metal. The common industrial processes are the so-called sulphate process and processes which demand concentrated sulphuric/hydrochloric acid and temperatures exceeding 1000 °C hence making the processes environmentally hazardous. Titanium dioxide particles are the common base material used in paints, pigments, and nanoparticles which are used in reducing toxicity of dyes and pharmaceutical drugs, wastewater treatment, reproduction of silkworm, space applications, food industries, and in dye-sensitized and perovskite solar cells. The iron component can be used to make magnetite, haematite, and zero-valent iron nanoparticles. We have developed a novel process to break down the strong ilmenite structure at 170 °C with the aid of mechanical energy using dilute hydrochloric acid enabling separation of titanium and iron components and silica impurities and converting them to useful nanomaterials.

Sri Lankan apatite

The apatite-bearing carbonate rocks are found in Eppawala, Sri Lanka and are generally known as Eppawala rock phosphate (ERP). Apatite occurs as massive, discontinuous bodies in a Precambrian, high-grade metamorphic terrain. When these apatite-bearing carbonate rocks are weathered they form economically important phosphate deposits. The naturally occurring Eppawala rock phosphate contains 28% to 42% phosphorus as P_2O_5 (Pitawala *et al*., 2002). Its usability as a phosphorus source in agriculture is limited by its low water solubility though the solubility can be increased using concentrated phosphoric acid or concentrated sulphuric acid to form triple superphosphate (TSP) and single superphosphate (SSP). Various researchers over several decades have extensively investigated these processes and satisfactory results were obtained. The finely ground raw phosphate product is used as a direct application as a fertilizer for perennial crops such as tea, rubber, and coconut. Studies have shown that the EPR is composed of an indiscriminate mixture of more water-soluble primary crystals and a less soluble secondary phosphate matrix rich in ferruginous and aluminous compounds, the latter being insoluble in water. Researchers have realized that the primary crystals can be easily separated from the rest of the components by mechanical separation, and they coined the term selectively mined primary crystals of Eppawala rock phosphate (SERP). The SERP has a high P_2O_5 content of 35% to 42% and much lower (less than 1%) trivalent metal oxides such as iron and aluminium oxides. The SERP is therefore water soluble and hence can be used directly for short-term crops such as rice and vegetables (Dahanayake *et al*., 1995). Eppawala apatite is impure hydroxycalcium phosphate with some hydroxyl groups exchanged for fluoride and carbonate ions. We have developed a method to convert this impure Eppawala apatite to pure hydroxyapatite nanoparticles for making metal and alloy prostheses used in orthopaedic transplants, which are biocompatible, osteo-integrating, non-toxic, and resistant to natural rejection by the human body.

Sri Lankan dolomite

Dolomite is basically a 1:1 mole ratio of $CaCO_3$:$MgCO_3$ though there can be small amounts of impurities such as silica. Both $CaCO_3$ and $MgCO_3$ are occur naturally in their pure form also in calcite and magnesite, respectively. The three minerals, dolomite, calcite, and magnesite are generally called limestone minerals. Dolomite is abundant in Sri Lanka and is found in Highland - Southwestern, Vijayan, and Wanni complexes. The limestone deposits are found in Anuradhapura, Habarana, Matale, Kandy, Ratnapura, Balangoda, Badulla, Bibile, Welimada, Ambilipitiya, Hambantota and Kataragama. Dolomitic mineral is the most abundant. The major use of dolomitic limestone is as quicklime and slaked lime for the building industry. Its minor uses are as a raw material in the ceramic and glass industry. Powders of dolomite are also used in the rubber and paint industries as filler material and to produce wall finishing materials. We have developed a methodology to separate the Ca and Mg carbonate components of dolomite and silica impurities, and then convert the Ca and Mg fractions to $CaCO_3$ and $MgO/Mg(OH)_2$ nanomaterials for various applications, as presented in a later section.

Sri Lankan clays

Clay is not considered a primary mineral, but it is a weathered product of various aluminosilicate minerals. Clays can be generally categorized as weathered hydrated aluminosilicate minerals. The major minerals present in clay are kaolinite, montmorillonite, and micaceous clay minerals. The major use of clay is for the ceramic industry, though there are many other applications such as in pottery. Clays are excellent materials to be used as clarifiers, absorption and adsorption materials, and in paper, paint, petroleum, ceramic, cement, adhesive, asphalt, food, and health-care industries. We have developed montmorillonite/ electronically conducting polymer nanocomposites for various technological applications as presented later.

Sri Lankan feldspar

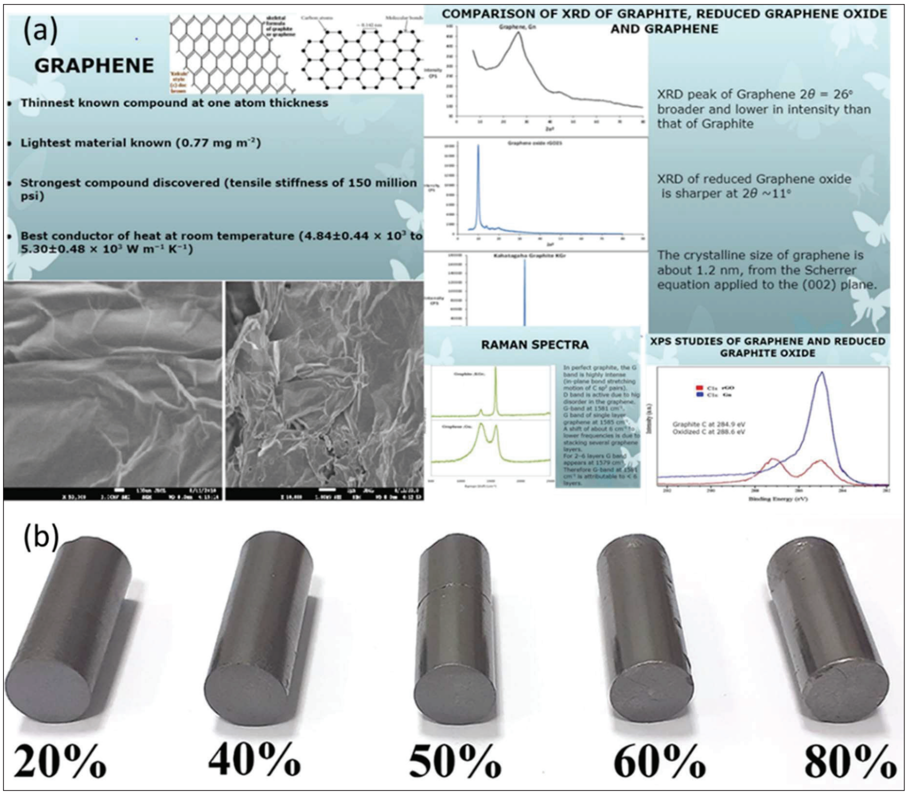
Feldspar is an aluminosilicate mineral containing potassium, sodium, and calcium inclusions. Feldspar deposits are abundantly found throughout Sri Lanka, particularly, in Rattota, Namaloya, Koslanda, and Balangoda localities. Feldspar's major use is as a refractory mineral in ceramic and glass industries. However, some feldspar deposits are rich in potassium with over 10% w/w natural abundance. We are currently developing methodologies to extract potassium from feldspar using microorganisms such as bacteria and fungi.

CONVERSION OF SRI LANKAN MINERALS TO VALUE-ADDED MATERIALS

Graphite to expanded graphite and graphene products

We have developed a chemical-free flotation method to purify Sri Lankan vein graphite. The purified graphite obtained was characterized by X-ray diffraction (XRD), scanning electron microscopy (SEM), transmission electron microscopy (TEM), infrared and Raman spectroscopic techniques, and by elemental analysis (Kumara *et al*., 2021). The silicate minerals with copper and iron that are present in raw samples have been removed via the flotation. Due to this, the carbon percentage has increased to 97% from 86% in the original mineral. This is a low-cost and environmentally friendly process for purifying natural vein graphite samples. We have also developed an acid mixture to delaminate graphite attached to pieces of rock of the graphite vein banks of the Bogala graphite mine, and selectively take it into the suspension to make expanded graphite and graphene products. The expanded graphite thus produced has an extremely high oil absorption capacity of 120 g of oil per 1 g of expanded graphite. This capacity is 20 times higher than that of biochar. The data obtained are summarized in Figure 2. In the conversion of graphite to graphene, graphite is first oxidized to graphene oxide (GO), and then reduced to produce reduced graphene oxide (rGO). Depending on the extent of oxidation followed by reduction, multi-layer graphene products are formed. In our research, the crystallite size of graphene is 1.2 nm. Graphene is the thinnest known material, made of only a one carbon atom thick layer, and is the lightest material known with the surface density of 7.710^{-4} g m^{-2}. Graphene is the strongest material ever discovered with a fracture strength of 130 GPa (Lee, 2008), the second- and third-order elastic stiffnesses of 340 N m^{-1} and –690 N m^{-1}, respectively, and a Young's modulus of 1.0 TPa. Graphene is highly thermally conductive, exhibiting a thermal conductivity of ~4000 W m^{-1} K^{-1} (Nair *et al*., 2008; Chen *et al*., 2010). The electrical conductivity of graphene is 10^8 S m^{-1} and is 1.68 times higher than that of copper.

Additionally, we have developed a technology to produce graphite electrodes using montmorillonite clay as the binder. The electrodes prepared are shown in Figure 2(b). These electrodes have conductivities of 27, 227, 454, 625, and 1250 S cm^{-1}, and are suitable not only for bulk electrolysis but also in electroanalytical chemistry in both aqueous and molten salt electrolytes (Karunadasa, 2019a)



- Thinnest known compound at one atom thickness
- Lightest material known (0.77 mg m^{-2})
- Strongest compound discovered (tensile stiffness of 150 million psi)
- Best conductor of heat at room temperature (4.84±0.44 × 10^3 to 5.30±0.48 × 10^3 W m^{-1} K^{-1})

XRD peak of Graphene $2\theta = 26°$ broader and lower in intensity than that of Graphite

XRD of reduced Graphene oxide is sharper at 2θ ~11°

The crystalline size of graphene is about 1.2 nm, from the Scherrer equation applied to the (002) plane.

In perfect graphite, the G band is highly intense (in-plane bond stretching motion of C sp^2 pairs). D band is active due to hig disorder in the graphene. G-band at 1581 cm^{-1}. G band of single layer graphene at 1585 cm^{-1}. A shift of about 6 cm^{-1} to lower frequencies is due to stacking several graphene layers. For 2–6 layers G band appears at 1579 cm^{-1}. Therefore G-band at 1581 cm^{-1} is attributable to < 6 layers.

Graphite C at 284.9 eV
Oxidized C at 288.6 eV

Figure 2: (a) Properties of graphene produced from Sri Lankan vein graphite. (b) The graphite electrodes prepared by using montmorillonite clay as the binder and firing the mixtures at 550 °C. The percentages shown are the percentages of graphite in the mixture used to prepare electrodes. (Reproduced with permission from Karunadasa, 2019a)

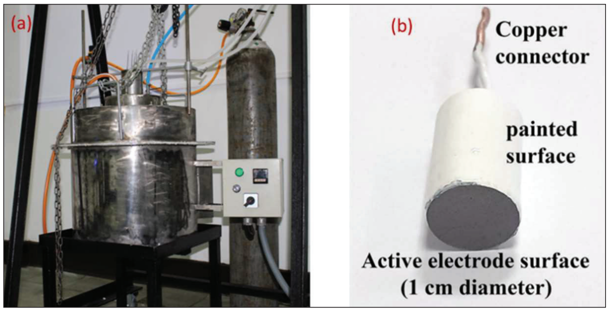


Figure 3: (a) The stainless electrolyser used to reduce silica to silicon at molybdenum anode and graphite cathode. (b) The graphite cathode fabricated. (Reproduced with permission from Karunadasa, 2018a)

Value addition to Sri Lankan quartz

We are developing an electrochemical process for the reduction of silica to silicon under inert atmosphere maintained with an argon flow, in molten $CaCl_2$ and/or a $CaCl_2$-NaCl salt mixture, at its eutectic point as shown in Figure 3(a). The stainless electrolyser was developed by fixing heat-stable tiling in the inside walls to maintain required the temperature without significant heat loss at a high temperature of 750 °C. The graphite anode shown in Figure 3 (b) was used and the cathode material used was a molybdenum wire which was wrapped around the silica pellets. At the molybdenum cathode, silica is reduced to silicon while producing oxide ions [Equation (1)].

At the molybdenum cathode:

$$\mathrm{SiO_2} + 4e^- \rightarrow \mathrm{Si} + 2\mathrm{O}^{2-} \quad ...(1)$$

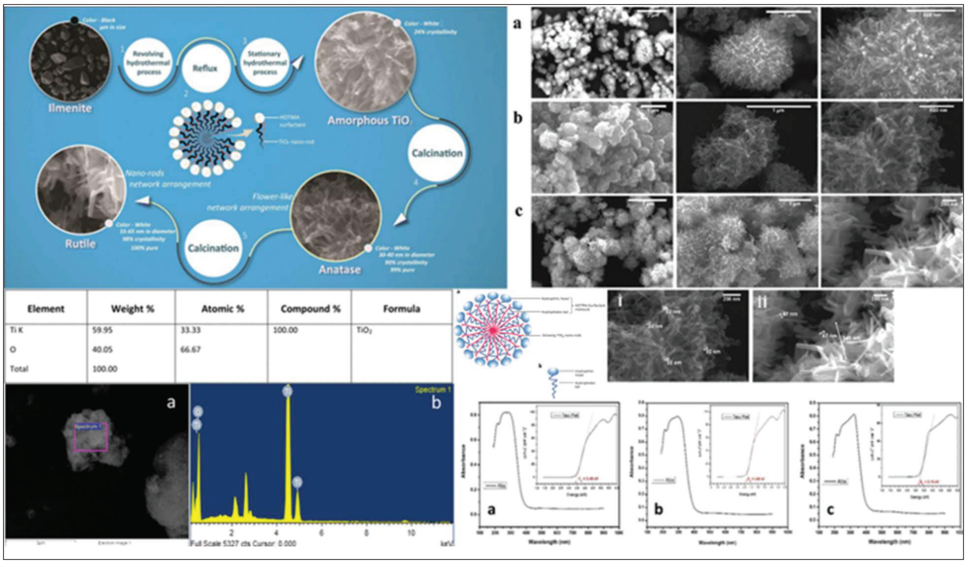
The ions produced at the cathode are diffused to the graphite anode and react with graphite oxidizing some C to CO and CO_2. To eliminate the evolution of CO_2, a molten $CaCl_2$-CaO mixture is used where CaO reacts with CO_2 forming $CaCO_3$. If an inert anode material is used instead of graphite, then the anodic oxidation process will be the oxidation of ions to O_2(g) as shown in Equation (2).

At an inert anode: $2O^{2-} \rightarrow O_2 + 4e^-$ ...(2)

This is an environmentally friendly process. This process is similar to the FFC Cambridge Process, and the electrolysis is carried out at an applied voltage of 2.3 V. The thermal decomposition of the calcium carbonate used was extensively studied using high-temperature P-XRD measurements (Karunadasa, 2019b). The relative stability of hydrated/anhydrous products of the calcium chloride used was also studied using high-temperature P-XRD (Karunadasa, 2018b). The composition, unit cell parameters and microstructure of quartz during phase transformation from α to β was examined by *in situ* high-temperature P-XRD (Karunadasa, 2018a). Having extensively characterized all the materials used, electrodes fabricated, and the electrolyser developed, we are currently investigating the conversion of silica micro-particle-based pellets to silicon via electrochemical reduction. All the apparatus shown in Figure 3 was fabricated locally at the Industrial Technology Institute (ITI) and the stainless-steel electrolyser can withstand high temperatures up to 1000 °C. Therefore, the electrolysis can be easily carried out at the melting temperatures of calcium chloride and its eutectic mixtures with other chlorides.

Value addition to Sri Lankan ilmenite

Ilmenite is composed of iron titanate with the formula unit $FeTiO_3$, and we have developed a mechanical method based on rotating autoclave to break down the ilmenite structure, separate the titanium and iron components, and convert them into phase-specific nanomaterials. This process happens at 170 °C, thus making it very low-cost. The final discharge contains only sodium chloride solution, which can also be recovered in a saltern. Therefore, the process is environmentally friendly also. We have applied for two NIPO patents and one WIPO patent, and two ACS Omega publications. Figure 4 shows schematic representation of the process of making phase specific TiO_2 nanomaterials and characterization of materials synthesized. As can be observed from atomic percentage data the nanomaterials contain only Ti and O in 1:2 atomic ratio thus confirming 100% purity of the TiO_2 nanomaterials prepared (Rajakaruna, 2020). When the separated titanium sol obtained was mixed with the surfactant hexadecyltrimethylammonium bromide (HDTMA) at a concentration above its critical micelle concentration and subjected to hydrothermal treatment in a closed system of an autoclave, a disordered phase of $Na_2Ti_3O_7$ was obtained. Since these particles are formed within the micelle structure, the product has a flowerlike morphology of rods emerging in all directions starting from one point. The as-synthesized TiO_2 nanoparticles are in an amorphous state that can be converted into over 99% pure anatase phase with 90% crystallinity and 100% pure rutile phase nanorods with 98% crystallinity by calcining at 350 °C and 650 °C, respectively. The direct band gaps of the three materials determined using Tauc plots are 3.40 eV, 3.60 eV, and 3.15 eV, respectively.



Element	Weight %	Atomic %	Compound %	Formula
Ti K	59.95	33.33	100.00	TiO_2
O	40.05	66.67		
Total	100.00			

Figure 4: Schematic illustration for the conversion of ilmenite to pure titanium dioxide nanomaterials and their characterization. (Reproduced with permission from Rajakaruna, 2020)

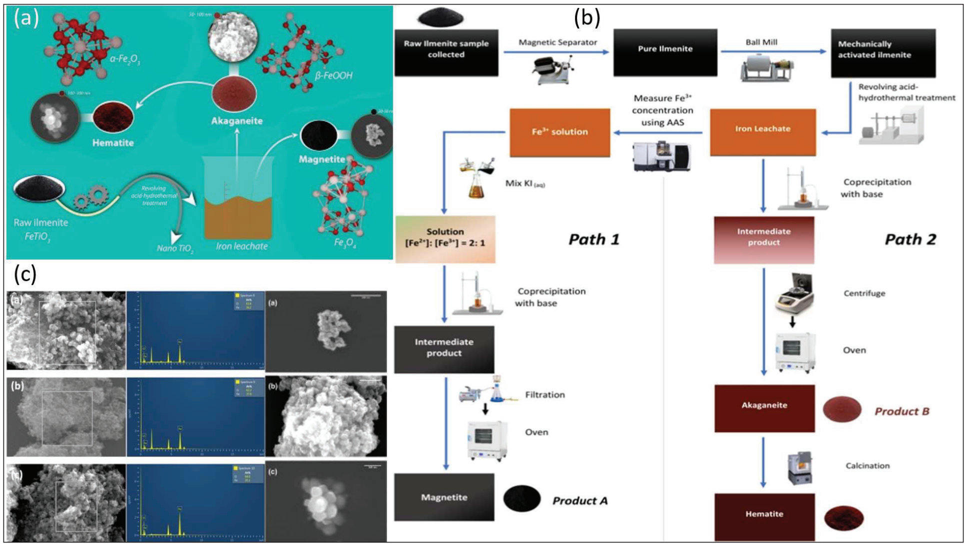


Figure 5: (a) Schematic illustration of the processes involved in converting iron component of the ilmenite to akageneite, magnetite and haematite nanomaterials, (b) the paths used and (c) characterization of the products obtained. (Reproduced with permission from Rajakaruna, 2021).

We have already demonstrated applications of titanium dioxide nanomaterials in dye-sensitized and perovskite solar cells and in many other application (Jayaweera *et al*., 2015, 2017; Ranasinghe *et al*., 2015; Senevirathne *et al*., 2016; Wanninayake *et al*., 2016; Kumarasinghe *et al*., 2019; Uthayaraj *et al*., 2019; Kumarasinghe *et al*., 2021).

When the titanium component was removed, the remaining iron component was converted into iron oxide (magnetite and haematite) and iron oxy-hydroxide (akageneite, β-FeOOH) nanoparticles (Rajakaruna, 2021). Figure 5 shows the process used and the characterization of the products formed. Figure 5(a) schematically shows the process for the conversion of the iron component into the akageneite phase of iron oxyhydroxide, and the magnetite and haematite phases of iron oxide nanomaterials. Figure 5(b) illustrates the paths used and 5(c) shows morphological and elemental characterizations. When both titanium and iron components are removed, the wastewater contains only sodium chloride that can be recovered in a saltern eventually releasing pure water to the environment. This is a very low-cost and environmentally friendly process that should be utilized to convert our natural ilmenite present in mineral sands into such highly value-added products demanded by many industries.

Value addition to Sri Lankan apatite

In our research, we have developed technologies to synthesize and to convert local apatite to pure hydroxyapatite nanomaterials. In the synthesis of pure hydroxyapatite nanomaterials, we used a calcium source, a phosphate source, and a base to provide hydroxyl ions (Somarathna *et al*., 2016; Gamagedara *et al*., 2018; Wijesinghe *et al*., 2019. For the conversion of local apatite to pure hydroxyapatite nanomaterials, we used urea as the fuel in the combustion method (Somarathna, 2016). In this method, powdered apatite was digested and combusted to produce calcium phosphate nanoparticles. The calcium phosphate nanoparticles thus produced were then hydrothermally treated to convert them into high purity hydroxyapatite (HA) nanoparticles [Figure 6(a)]. The HA nanoparticles were characterized using XRD, FT-IR spectroscopy, SEM [Figure 6(b)] and TEM [Figure 6(c)]. The synthesized HA nanoparticles are nontoxic according to the cytotoxicity results, which confirmed their potential usage in biomedical applications. As such, we developed technology to chemically attach HA nanoparticles to stainless steel and titanium alloy (Ti-6Al-4V) prosthesis materials [Figure 6(d)]. The prostheses thus produced were evaluated for their toxicity, biocompatibility, osteo-integration, and corrosion in the body fluidic environment. Having obtained satisfactory results, two such custom-made prostheses were manufactured and transplanted into needy patients [Figure 5(e)] (Rajapakse, 2016; Wijesinghe, 2014a).

Value addition to Sri Lankan dolomite

Dolomite is ~50:50 $CaCO_3$:$MgCO_3$ with some silica impurities. When calcined at a temperature above 350 °C, $MgCO_3$ undergoes decomposition according to the reaction $MgCO_3 \rightarrow MgO + CO_2$ with an enthalpy of reaction, ΔH_{dec} of $+118$ kJ mol^{-1}. However, $CaCO_3$ requires a higher temperature for the decomposition; it starts slowly at 650 °C and occurs fast beyond 750 °C, with a ΔH_{dec} of $+178.1$ kJ mol^{-1}. Therefore, when dolomite is heated above 750 °C, both $CaCO_3$ and $MgCO_3$ undergo rapid decomposition forming CaO.MgO that can be converted to $Ca(OH)_2$ and $Mg(OH)_2$ upon hydration. The solubility product constant of $Mg(OH)_2$

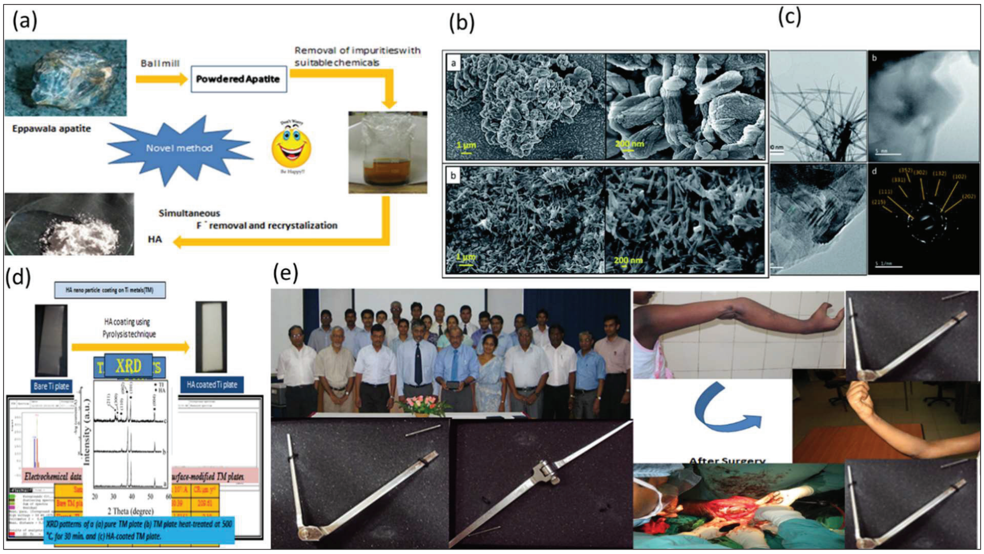


Figure 6: (a) Schematic representation of the combustion process used to convert Eppawala apatite into pure hydroxyapatite nanomaterials; (b) SEM and (c) TEM images of the final product; (d) coating of the hydroxyapatite nanomaterials on to titanium prosthesis material and characterization and (f) orthopaedic transplantation of the custom-made prostheses in needy patients. (Reproduced with permission from Wijesinghe *et al*., 2014b and Rajapakse *et al*., 2016)

is 1.2 × 10^{-11} whereas that of $Ca(OH)_2$ is 5.5 × 10^{-6}. Due to the comparatively high solubility of $Ca(OH)_2$ in water, we found that when a mixture of CaO and MgO is stirred in a sucrose solution, CaO selectively dissolves but MgO is converted to insoluble $Mg(OH)_2$ and remains as a precipitate together with silica impurities. Therefore, we were able to separate the Ca component into the aqueous solution. The Mg component was separated from silica impurities by dissolving $Mg(OH)_2$ in dilute HCl and thereby we were able to separate Ca and Mg components and silica impurities. The calcium component was converted to phase-specific $CaCO_3$ nanoparticles and the magnesium component to MgO and $Mg(OH)_2$ nanoparticles. The nanoparticles synthesized were used to encapsulate several anticancer drugs for targeted delivery and slow release to eliminate cytotoxicity of the drugs to healthy cells and to reduce dosage, while increasing the bioavailability and efficacy of the drugs. The laboratory studies were extended to scaling up research at the Department of Chemical and Process Engineering, Faculty of Engineering, University of Peradeniya, under the National Science Foundation's Technology Grant No. TG/2016/Tech-D/02, where a spinning disc reactor was developed to synthesize $CaCO_3$ nanoparticles on a large scale. In this research, superhydrophobic calcium carbonate nanoparticles were also prepared for various industrial applications. The superhydrophobic $CaCO_3$ nanoparticles were stabilized with polyacrylate polymer, and the composite was used as a water-repellent wall paint (Mantilaka *et al*., 2014a; 2014b; 2014c; Wijesinghe *et al*., 2014b; Abeywardena *et al*., 2020; Gunathilake *et al*., 2020).

We have also developed methods to prepare porous vaterite spherical nanoparticles and magnesium oxide nanoflakes starting from dolomite. These nanomaterials, and also many other oxide nanomaterials were used to encapsulate anticancer drugs such as cisplatin, doxorubicin, paclitaxel, and vinblastine for targeted delivery and slow release only at the cancerous sites to eliminate cytotoxicity of the drugs to healthy cells, to reduce wastage of the drug due to toxic adduct formation, and hence to reduce the dosage requirement while increasing the bioavailability and efficacy of the drugs (Dunuweera & Rajapakse, 2017; Weerasuriya *et al*., 2017; Ranathunge *et al*., 2019; Wimalasiri *et al*., 2021)

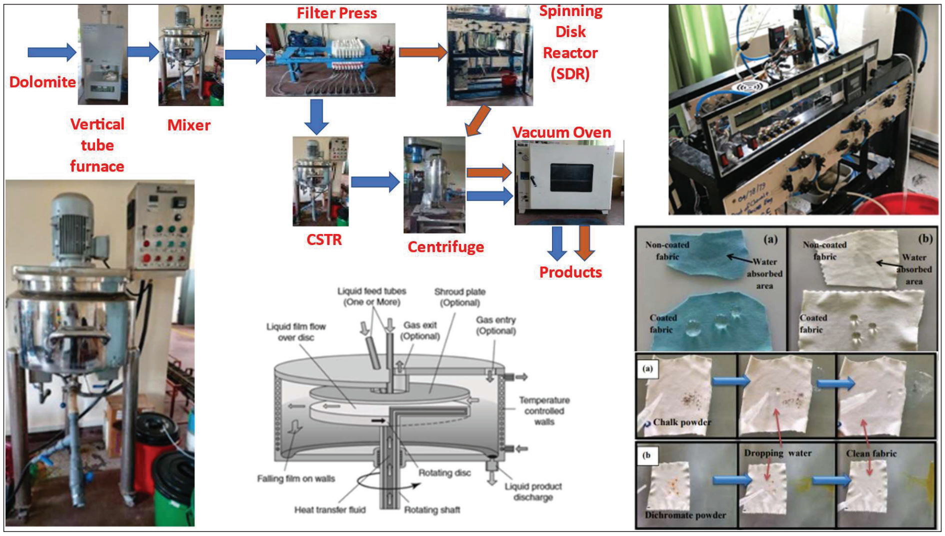


Figure 7: (a) The process flow demonstrating conversion of dolomite to final calcium carbonate products; (b) the Continuous Stirred Tank Reactor (CSTR); (c) the mechanism of the spinning disc reactor; (d) superhydrophobic calcium carbonate produced and demonstration of the stain resistance of superhydrophobic calcium carbonate attached cotton fabric. (Reproduced with permission from Abeywardena *et al*., 2020). Scaling up research is led by Prof. D.P.P. Karunaratne, Prof. H.M.T.G.A. Pitawala, Prof. R.M.G. Rajapakse, Dr Aruna Manipura and Dr M.M.M.G.P. Mantilaka.

Value addition to Sri Lankan montmorillonite clay

Montmorillonite (MMT) is an aluminosilicate clay with negatively charged clay sheets formed due to isomorphic substitution of aluminium by silicon and magnesium or other divalent cations. The interlayer spaces accommodate hydrated cations to counterbalance the excess negative charge. These cations are usually Na^+ in Na^+-MMT, and these cations are easily exchangeable with other cations such as H^+ to form H^+-MMT or organic cations to form organoclay. We made use this exchangeability to incorporate anilinium ions within the interlayer spaces of MMT clay and to chemically polymerize it using an oxidant such as persulphate or iron (II), to form polyaniline (PANI) in its conducting state. The PANI-MMT clay-polymer nanocomposites thus formed were extensively characterized. Additionally, we made PPY-MMT, PEDOT-MMT nanocomposites where PPY and PEDOT stand for polypyrrole and polyethylenedioxythiophene, respectively. Various oxidants were used instead of the two oxidants quoted above. There, we used oxidizing metal cations such as Ag^+, Hg_2^{2+}, Pd^{2+}, etc. to form nanocomposites of metal atoms/ECP/MMT where ECP stands for ‘electronically conducting polymer,’ such as PANI, PPY, and PEDOT. These novel nanocomposites were found to be excellent cathode materials for alkaline and phosphoric acid fuel cells to replace expensive platinum by the low-cost metal atoms/ECP/MMT nanocomposites (Krishantha *et al*., 2005, 2006; Rajapakse *et al*., 2005, 2006; Bandara *et al*., 2016; Senevirathne *et al*., 2016). The MMT clay is also found to be an excellent material for improving mechanical properties such as abrasion resistance of gloves produced in the Sri Lankan glove industries.

Value addition to Sri Lankan feldspar

Sri Lankan feldspar is rich in potassium with at least 10% by mass natural abundance in the aluminosilicate clay material. We are currently working on a green method to extract potassium present in clay using microorganisms such as bacteria and fungi. We have found that *Aspergillus* fungi species isolated from the wastewater of cattle farms can release potassium from feldspar. We are working on developing organically produced potassium fertilizer from our natural clay minerals.

Current Market Analysis for Nanomaterials Produced from Sri Lankan Minerals and the Products Developed

The global market for prosthetics and orthotics was around USD 6.11 billion in 2020. It is expected to reach USD 6.39 billion in 2021, and also expected to grow at an annual growth rate of 4.2% and reach USD 8.6 billion by 2028 [Prosthetics & Orthotics Market Size, Share & Trends Analysis Report by Type (Orthotics, Prosthetics), By Region (North America, APAC), And Segment Forecasts, 2021 - 2028, n.d.]. The global oil spill management market size was USD 131.16 billion in 2015. Based on estimates done in 2015, the market size value in 2020 was USD 159.62 billion with a revenue forecast in 2025 of USD 177.63 billion when calculated at the growth rate of CAGR of 4.0% from 2018 to 2025 (Oil Spill Management Market Size & Share Report, 2018-2025, n.d.-a). The global Nano TiO_2 market was USD 3.4 billion in 2016. With an expected growth rate at CAGR of 20.7% in the next six years, the projected global market in 2022 is USD 16.862 billion (Oil Spill Management Market Size & Share Report, 2018-2025, n.d.-b). The pigment only market for iron oxide/iron nanoparticles in 2021 was USD 2.3 billion. With a growth rate CAGR of 4.7% from 2021 to 2028, the revenue forecast for 2028 is 3.1 billion (Iron Oxide Pigments Market Size | Industry Report, 2021-2028, n.d.). The global silicon metal market size will increase to 9170 million USD by 2025, from 6520 million USD in 2018, at a CAGR of 4.4% during the forecast period. In this study, 2018 has been considered as the base year and 2019 to 2025 as the forecast period to estimate the market size for silicon metal (Global Silicon Metal Market Size In 2021: Growth by Forthcoming Developments, Industry Scope, Opportunity, Business Strategy and COVID-19 Market Scenario | Report by Industry Research Biz - MarketWatch, n.d.). These are some of the examples of the billion-to-trillion-dollar global market available for the materials and devices made starting from local minerals. It is unfortunate that the responsible authorities in Sri Lanka are not considering these developments and taking steps to develop the national economy of the country. The political leadership we had since we obtained independence in 1948 never had a right vision to develop the national economy of the country and as a consequence Sri Lanka is now a country where survival of the nation has become problematic. If our natural resources can be managed in a proper manner by adding value to them then Sri Lanka could become one of the developed countries in the world.

CONCLUSION

Although Sri Lankan researchers have developed novel technologies to add high value to local minerals through low-cost processes and invented novel devices and materials, the incorporation of research output in the industrial processes and integrating scientific advances in the export market are lacking. As this review highlights, Sri Lanka is a country with naturally abundant high-quality minerals, and their processing to improve purity and to convert them to highly value-added products has been limited to research publications and patents without utilizing these developments in real world applications. This is due to short-sighted political leadership of the country throughout its history since receiving independence in 1948. It is time that the political leadership of the country realized the importance of adding value to local minerals at least by understanding the market research described in section 3. If the country had a leadership with a correct vision and mission, the country could have been among the most developed countries of the world. Currently, we are among the poorest nations of the world despite having such natural treasures and devoted academics and researchers who have contributed enormously towards adding value to these natural resources. It is emphasized that the country should gear towards adaptation of scientific developments in order to stand along as a developed nation.

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REVIEW

COVID-19 pandemic: lessons for global health security

JSM Peiris*
School of Public Health, The University of Hong Kong, Hong Kong.

INTRODUCTION

The COVID-19 has been the worst pandemic in over a hundred years, impacting on the global economy and psyche, far beyond its direct health impacts. In terms of direct virus associated mortality overall, COVID-19 has led to fewer deaths globally than the 1918 "Spanish Flu." It is estimated that the 1918 pandemic (which went on for a few years beyond 1918) was associated with around 50 million deaths world-wide, at a time when the global population was around 1.8 billion people (much smaller than the current global population of 7.9 billion), and thus, the 1918 pandemic killed 2.7% of the global population. It had a major societal impact in many economically advanced countries with many people retaining memories of that event decades later. The Spanish Flu does not appear to have impacted the psyche of the Ceylonese population, but recent studies suggest that approximately 6.7% of the population lost their lives to this pandemic in Sri Lanka (Chandra & Sarathchandra, 2014).

In contrast to the Spanish flu, The World Health Organization (WHO) confirmed COVID-19 deaths as of end of 2021 is approximately 5.5 million, with estimates of COVID-19 associated excess-mortality suggesting that COVID-19 deaths are 2–4 times higher than confirmed deaths (Adam, 2022). Even so, overall global mortality is of the order of 0.28% of the global population, ten-fold lower than the impact of the 1918 Spanish flu. Nevertheless, COVID-19 appears to have had an even greater economic and social impact in most parts of the world, likely because of the globalized economy, supply chains and communications that make the world more inter-dependent than in 1918. Although the full economic impact of COVID-19 cannot yet be estimated, it has been estimated to lead to a reduction of 7.3% of GDP growth overall, with the greatest impact (-8.7%) in middle income countries (Sanchez, 2021). These assessments do not capture the adverse impacts on widening inequalities overall, and particularly so for women, marginalized and vulnerable populations. Social impacts are even more difficult to quantify, ranging from lost educational opportunities and loss of social-skill development in children and long-term psychological and behavioural impacts on a whole generation.

Since neither specific antiviral drugs nor vaccines were available at the early stages of the COVID-19 pandemic, the main counter-measures available were the same "public health and social measures" (PHSM) that were used during the 1918 pandemic. But virologic testing to identify cases for treatment and isolation was also available, an option not available in 1918. Indeed, it was the published data and experience on the efficacy of public health interventions in 1918 that informed the early response to the COVID-19 pandemic in 2020. It is interesting to read some of the recommendations from 1919, viz. avoid needless crowding, smother your coughs and sneezes, open the windows (i.e. ventilation), wash your hands ("your fate may be in your own hands") (Soper, 1919). Similarly, the problems faced in pandemic control in 1918 were also similar, public indifference ("people do not appreciate the risks they run"), "It does not lie in human nature for a man who thinks he has only a slight cold to shut himself up in rigid isolation as a means of protecting others," and "the disease may be transmissible before the patient himself is aware that

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* malik@hku.hk; https://orcid.org/0000-0001-8217-5995

he is attacked" (Soper, 1919). It is humbling that this analysis, written over one hundred years ago, at a time when the causative agent of the 1918 pandemic was yet unknown and the properties of viruses not understood, still remains true now, in a much more technologically advanced setting. In contrast to 1918, the past two years have seen scientific evidence to prove the effectiveness of some of the non-pharmaceutical interventions such as wearing of masks and other public health and social measures (Haug *et al*., 2020; Andrejko *et al*., 2022). It is also a striking contrast with COVID-19 that safe and vaccines were developed and deployed within a year of the emergence of the pandemic in 2020, testament to the scientific and technological advances in the past one hundred years. Large clinical trials identified generic therapeutic interventions (e.g. dexamethasone, IL6 receptor antagonists) that can reduce mortality, and importantly, also identified many that did not (e.g. hydroxychloroquine). Although antiviral therapeutics took longer to develop and be validated, a number of options are now becoming available by early 2022.

Given the impact of the COVID-19 pandemic, it is important to understand the lessons of its emergence and the global response to it, so that we are better prepared to prevent, pre-empt and mitigate similar events in the future. Future pandemics are inevitable, and we need to accept that such pandemics may be even more severe than COVID-19. Key measures that need to be taken have been highlighted in the report of The Independent Panel for pandemic preparedness and response (Table) (The Independent Panel, 2021).

Table: Recommendations to ensure that future outbreaks do not become pandemic:

a. Invest in preparedness now to prevent the next crisis
b. Raise new international financing for pandemic preparedness and response
c. A new agile and rapid surveillance system
d. Strengthen the independence, authority and financing of WHO
e. Elevate pandemic preparedness and response to the highest level of political leadership
f. National pandemic coordinators have a direct line to Heads of State or Government
g. Establish a pre-negotiated platform for tools and supplies for emergency health responses

I will summarize the lessons learned from COVID-19 and actions needed for better pandemic responses in the future, pertaining in particular, to points a, b and c of the Independent Panel Recommendations by addressing three inter-related questions. A. Was this pandemic or its severity unexpected? B. Why do we continue to face novel emerging infectious diseases and what can we do about it? C. How can we enhance capacity for surveillance and response?

Was this pandemic or its severity unexpected?

This pandemic was expected, predicted and the potential impacts and challenges highlighted. Even the impact of a pandemic on global supply chains and the adverse impact of disinformation on pandemic response was predicted (Osterholm & Oashaker, 2017). But there was a failure of global leadership to make the investments and preparations needed for a more effective response. What we need going forward, is a dramatic change in prioritization from political and business leaders of preparedness for pandemic threats and their impacts, together with a commitment to develop countermeasures and enhancing resilience of health care systems, economies and societies.

Major advances have been made in combatting infectious diseases over the past hundred years or so, with a scientific understanding of their causes (the germ theory of infectious disease) and modes of development of treatments (antimicrobials) and prevention (vaccines). Feared infectious diseases of the past such as diphtheria or measles have been made preventable and others eradicated (e.g. smallpox) or are close to eradication (e.g. polio). However, novel infectious disease threats continue to emerge. In the past few decades, we have faced HIV/AIDS, Avian influenza viruses H5N1 and H7N9, SARS, the 2009 "swine flu" pandemic, Ebola and Zika, prior to COVID-19. In the words of a recent WHO report, the emergence of antibiotic resistance threatens to lead to a "post-antibiotic era, in which common infections and minor injuries can kill", a scenario "far from being an apocalyptic fantasy – is instead a very real possibility for the 21st century" (WHO, 2014). These emerging infectious disease outbreaks have individually cost billions of US dollars (SARS in 2003–30-50 billion US$; Avian flu H5N1 from 2004-2008 30 billion US$; Ebola in West Africa 53 billion US$). The risk of a sarbecovirus (SARS-CoV related viruses) posing a pandemic threat was identified 5 years prior to the emergence of COVID-19, with the discovery of coronaviruses in bats, distinct but related to SARS-CoV-1, that could infect human cells (Menachery *et al*., 2015). A bat coronavirus also caused an outbreak of diarrhoea in swine in China (Zhou *et al*., 2018).

However, the investments made to address these threats have not matched their observed impact. It appeared that mankind managed to dodge each of these events, as they appeared to be contained (e.g, SARS in 2003; Ebola in 2015) or be relatively mild ("swine flu" pandemic in 2009). Given the frequency with which these zoonotic diseases were emerging, it was inevitable that, sooner or later, one with greater consequence would emerge. That was COVID-19. It is important to recognize that this may not in fact, be the most severe pandemic of the 21st Century. Thus, it is imperative that we learn the lessons from COVID-19, so that we are much better prepared for future pandemic threats.

Why do we continue to face novel emerging infectious diseases and what can we do about it?

The reasons for the increasing frequency of emergence of novel infectious disease are well established and mainly pertain to human activities, viz. intensive livestock production methods (e.g. 80 billion livestock consumed annually, limited genetic diversity of livestock-lines with aim of high levels of production makes it easier for a virus to spread), wild-game animal trade, the bush meat trade, pet animal trade, human population growth and urbanization, antibiotic abuse, breakdown of public health systems, wars, ecological degradation and deforestation and climate change. Microbial factors such as mutation and adaptation also play a role but these have remained the same over millennia. It is the changes in human-drivers that contribute to the progressive increase in emerging infectious diseases over the past 50 years (Jones *et al*., 2008). For example, the SARS outbreak in 2003 emerged via the wild game animal trade (Guan *et al*., 2003). Recent studies add credence to the likely hood that SARS-CoV-2 also arose in a similar manner, from the Huannan seafood market in Wuhan, which also appeared to be selling wild game animals, either live or freshly slaughtered for human consumption (Cohen, 2022). This recognition led to the closing of game animal markets in Southern China in 2004 and prevented the re-emergence of SARS, but this was not extended to other parts of China and Southeast Asia in a sustainable way. If it had, it is very likely that COVID-19 may not have emerged. A recent study of the wild game animal trade in China identified 102 mammalian-infecting viruses, with 65 described for the first time. Twenty-one viruses were considered as potentially high risk to humans and domestic animals (He *et al*., 2022).

The trade and consumption of bush meat is responsible for the emergence of Ebola outbreaks in Africa. The trading of live poultry via whole-sale and retail markets and the sale and consumption of live poultry contributes to the emergence of zoonotic avian influenza A viruses such as subtypes H5N1 and H7N9 (Peiris *et al*., 2016). Understanding the pathways of virus emergence can allow interventions that reduce zoonotic and pandemic risk, an example of "risk reduction at source". For example, studies in Hong Kong demonstrated that intermittent closure of live poultry markets (market "rest-days") or even ensuring that the poultry market is emptied of poultry overnight, markedly reduced the maintenance and amplification of avian influenza viruses in these live poultry markets and reduced zoonotic risk. Our studies also show that separation of aquatic and terrestrial poultry, through the wholesale and retail marketing chain, will reduce the emergence of zoonotic threats such as avian influenza H5N1 and H7N9 (Peiris *et al*., 2016).

The pet industry involves the trade of animals across international borders, often involving wild-animal species and even endangered species. For example, this contributed to an outbreak of monkeypox in the USA (Reed *et al*., 2004) and the importation of infected pet hamsters from Europe led to the introduction of the Delta variant of SARS-CoV-2 into Hong Kong in January 2022, at a time when that variant was not present in Hong Kong (Yen *et al*., 2022). The international pet trade is massive; for example, 1.5 billion live-animals were imported into the USA during the period 2000–2009 (Smith *et al*., 2009). It is estimated that change of land-use and environmental habitat for expanding agriculture led to the emergence of Argentine haemorrhagic fever. The misuse and abuse of antibiotics as well as its use as a growth promoter in animal husbandry contributes to increase in antibiotic resistance. Climate change, as well as being inherently disastrous for planetary health, is contributing to the increases of vector-borne diseases. These "man-made" changes lead to planetary dysbiosis and are increasing the rate of emergence of new zoonotic diseases as well as having other undesirable consequences for the sustainability of species diversity, and even the survival of our own species, on planet earth. Given the multi-factorial and multi-disciplinary nature of the challenge, it is imperative that our response should also involve, human health, animal health and the environmental sciences, in a "One Health" response to these challenges.

Enhancing capacity for surveillance and response

Following the SARS epidemic of 2003, it was realized that a novel and unusual epidemic anywhere in the world could be a threat everywhere. In response to this, the WHO developed the International Health Regulations (IHR) which has been ratified by most countries in

2007. This is a binding agreement upon 196 states and the countries are expected to develop capacity to a) detect and report unusual and unexplained outbreaks of disease, b) develop capacity to respond, c) establish effective mechanism for surveillance, and d) develop public health capacity to respond to disease outbreaks when and where they occur. However, a decade later, it was realized that less than half of the countries in the world were confident of achieving these aims, even on their own self-assessment. It was clear that capacities for surveillance, epidemiological analysis, diagnosis and response were far from what is required to meet obligations under IHR. Even those countries that appeared to be well prepared, failed the test of responding adequately to the COVID-19 pandemic. For example, the Johns Hopkins University Global Health Security Index ranked the USA as the best prepared country to respond to a future pandemic. However, by July 2020, with 5% of the global population, it had 25% of confirmed cases worldwide and per-capita deaths were ten times higher than in Europe, with many East Asian countries responding to the pandemic more effectively than either the USA or Europe. As the Independent Panel makes clear, this delayed and inadequate response was not due to a lack of early warning but rather because too many "Developed Countries" under-estimated the risks, even after the WHO declared a Public Health Emergency of International Concern by the 30th January 2020, i.e. within 30 days of hearing about the new outbreak of pneumonia in Wuhan, China. It is clear that all countries need to honestly and realistically learn the lessons of COVID-19 because that would be the best to improve capacity to respond in the future. This includes strengthening capacity for laboratory diagnostics, epidemiology and a science-led leadership. The need for a "One Health"-based response capacity has already been emphasized above. Surveillance should be focused on unusual outbreaks of disease in both humans and animals (both wild and domestic) with active investigation of spill-over of infections across species.

Pre-emptive development of countermeasures

Methods for vaccine development of influenza are well known, but it takes over 8 months to develop and distribute a vaccine for a novel pandemic influenza virus. However, influenza pandemics move much faster than vaccine development and vaccines were not in time to mitigate the first wave of the 2009 pandemic of H1N1 influenza (Monto & Webster, 2013). Therefore, a system of proactive surveillance of influenza viruses at the animal-human interface, together with risk assessment of these viruses, has been implemented which can lead to development of pre-pandemic vaccines in advance of the emergence of an epidemic or pandemic (WHO, 2020). Both surveillance and risk assessment tools are now well established for zoonotic and pandemic influenza viruses, with global surveillance data being collected, collated and risk-assessed twice yearly at WHO influenza vaccine strain selection meetings. What is needed is for the global influenza virus program model to be expanded to cover the emergence of SARS- CoV-2 variants, and to cover emerging respiratory viral threats for the future.

In spite of repeated warnings from the scientific community of the threat from coronaviruses from bats, appropriate counter-measures (pre-emptive development of vaccines and antivirals) were not developed, because the coordinated global investment was not forthcoming. In fact, there had been considerable efforts to develop a vaccine for SARS soon after the outbreak in 2003 (Kam *et al.*, 2007) (Gillim- Ross & Subbarao, 2006), but research funding for these efforts rapidly waned with the effective containment of that outbreak. Thus, when COVID-19 emerged in early 2020, the pre-emptive preparedness that should have been in place was sadly lacking.

The pharmaceutical industry, which conventionally develops drugs and antivirals, cannot be expected to invest in development of counter- measures to emerging viruses that may or may not cause an outbreak in future, because such a model is not commercially viable (i.e. a particular virus may never emerge to transmit between humans). Clearly, such investment has to come from Governments and International agencies. This was not forthcoming, until 2017, when a Government-Private partnership, the Coalition for Epidemic Preparedness Initiative (CEPI), was launched at Davos 2017, as the result of an emerging consensus that a coordinated, international and intergovernmental plan was needed to develop and deploy new vaccines to prevent future epidemics, an initiative funded by the Governments of Norway, Japan, Germany and India, together with the Bill and Melinda Gates Foundation and the Wellcome Trust (CEPI). At its inception, it identified pandemic threat disease, including SARS, MERS, Ebola, Rift Valley Fever, Chickungunya, Nipha, Lassa fever and "disease X." If this initiative had a longer time to run, we would have been much better prepared to face COVID-19. As part of this program on emerging viral disease threats, vaccine development was initiated for MERS coronavirus (MERS-CoV). One of the earliest initiatives funded was to University of Oxford and Janssen Pharmaceutical, to fund a vaccine against Lassa and MERS-CoV using a similar adenoviral vaccine viral vector technology. Some of the developments arising from the MERS-CoV vaccine development provided a foundation for the University of Oxford team when they rapidly changed direction to the newly emerged SARS-CoV-2 coronavirus. As COVID-19 emerged, CEPI rapidly changed focus toward SARS-CoV-2 development and also was instrumental, with WHO, in setting up the COVAX facility, to facilitate global distribution of COVID-19 vaccines. This was, and is, a farsighted initiative that took initial steps to respond to the threats posed by emerging viral pathogens. If these steps had been taken earlier, by more organizations and on a larger scale, the world would have been much better prepared for COVID-19. The recent "100 days mission" launched by CEPI to fund development of vaccine technologies that can form the basis for even more rapid development of vaccines is to be applauded.

One of the lessons from COVID-19 must be to scale up initiatives such as CEPI, to address, not only vaccine development, but also, to address the challenge of equitable distribution of vaccines. COVID-19 vaccines were both one of the triumphs and disappointments of the pandemic SAGA. On one hand they were developed, licensed and deployed faster than most people believed possible and were effective and safe. But although over 8 billion vaccine shots have been given worldwide, sufficient to vaccinate all the priority groups needing vaccine world-wide, a large part of the less developed world remains unvaccinated. In the developed world, in spite of adequate vaccine supplies to give two, three of even four doses of vaccine per individual, conspiracy theories and rumours derailed vaccination campaigns. Taken together, these have resulted in thousands of needless deaths world-wide.

Vaccine inequity involved intellectual property issues and also involved the lack of vaccine manufacturing capacity in large parts of the world, e.g. Africa, together with the infrastructure to mount a rapid vaccination campaign in some parts of the world with associated cold-chain facilities. It will be important to address these gaps for the future. This also involves a greater investment in bio-medical research and in judicious efforts to set up capacity of local vaccine manufacturing in countries such as Sri Lanka. Indeed, the speed and collaborative nature of biomedical research during COVID-19 were one of the positive aspects to emerge from the pandemic, with vaccines being developed within a year, therapeutic interventions identified and rapid sharing of data on the emergence of novel viral variants (e.g. South Africa).

There are also other social issues that we need to be better prepared to counter. For example, we need better strategies to counter the significant spread of misinformation via social media that seriously cripples public health and control measures, (for example, false anti-vaccine propaganda). It became clear that the relative emphasis of "individual" rights vs. the "collective good" became a major tension in pandemic response in some so-called "developed countries".

CONCLUSION

Having just come through the heavy impacts of the COVID-19 pandemic, it would be a travesty if we do not learn its lessons and prepare to respond better, to future pandemics. The responses we take toward better pandemic preparedness are linked to our response to the other existential crises we face, such as the biodiversity loss and climate change. We need to move towards a trajectory for "economic development" that is not solely driven by "Gross Domestic Product". As Hinchliffe *et al*. (2021) recently eloquently put it, "The politics of planetary public health demand a shift away from (relying exclusively on) the biomedical, technological, and behavioural fixes and the economic imperatives of market capitalism, towards forms of governance and organisation where the health of the planet is assessed openly in terms of its equitability and sustainability".

Conflict of interest

The author has no conflicts of interest to declare.

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REVIEW

The road to precision cancer therapy – history and strategies

S Thiagalingam[1,2*]
Departments of Medicine (Biomedical Genetics and Cancer Center)[1], and Pathology & Laboratory Medicine[2], Boston University School of Medicine, 72 East Concord Street, E200, Boston, MA 02118, USA.

Summary: Human cancer has been recognized for thousands of years as a leading cause of death with complex pathophysiology and remains as the most challenging disease to achieve curative therapy. Selecting the right treatment for cancer patients based on molecular diagnostics continues to evolve. Recent explosion in the knowledge of molecular genetics, epigenomics, cellular biology and immunology of cancer has influenced the development of targeted therapies for specific tumour types as well as for the individual patients. Chemotherapy has remained as the backbone of cancer therapy, but limited response rates, side effects and resistance have increasingly shifted the focus on approaches to harness targeted therapy directed to specific molecular alterations at the intracellular genomic and epigenomic levels and the microenvironment which includes extracellular regulatory molecules and surrounding cells. Immunotherapies are designed to interfere with the immune escape mechanisms of cancer cells such as negative immunologic regulators (checkpoints) and/or by stimulating the patient's adaptive immune system against specific tumour antigens. Inherent complexity and heterogeneity of tumours as depicted by the multi-modular molecular network (MMMN) cancer progression model suggest that most cancers may not be treatable with just monotherapy and would require a combination of therapies, both traditional and targeted therapies as well as immunotherapies to lengthen the patients' life. The availability of novel technologies and generation of large-scale oncology data from multiple sources of analysis including genomics has created a promising environment for revolutionizing cancer diagnosis, drug discovery, treatment and prevention. Advanced computational algorithms are currently being developed for automatically interpreting input data to assist inter-professional teams to provide calibrated recommendations with minimal toxicities to help the physicians to make effective therapeutic decisions.

Keywords: Artificial intelligence (AI), computational algorithms, genome, multi-modular molecular network (MMMN), oncogenes, precision cancer therapy, targeted cancer therapy, tumour suppressor genes.

History of cancer therapy

Human cancer has been described as early as 2500 to 1500 BC in ancient Egyptian papyri and hieroglyphic inscriptions referring to the soft tissue and fatty tumours involving the skin, breast, stomach, rectum and uterus. Egyptians made attempts to treat the cancer patients using surgery, religion and magic/charm. They also differentiated benign as well as malignant tumours and found limited success with surgical resection of surface tumours while acknowledging lack of curative therapy (American Cancer Society; National Cancer Institute). The great Greek physician Hippocrates (460-360BC) who is considered as the father of medicine, first referred to cancers as karkinoma (Carcinoma) as it looked like karkinos (Greek for crab) because of the swollen blood vessels around the central body/lump resembling the claws and legs of a crab. Hippocrates promoted the belief that an imbalance of the main four body fluids or humors (blood, phlegm, yellow bile and black bile) caused diseases. He suggested the overrepresentation of black bile in the flesh as the cause and suggested diet, rest, and exercise to address this imbalance. He advocated surgery if the carcinoma was on the surface and not too deep. The most prominent successor of Hippocrates was Claudius Galen (AD 130 to 200). He used the word oncos (Greek for mass or swelling) to describe tumours, which resulted in the term for studying and treating cancer, oncology (Papavramidou *et al*., 2010). Galen considered that the patients are incurable after a diagnosis of cancer; however, he did write about surgical cures for breast cancer if it could be completely removed at an early stage. For many centuries, while there was progress in medicine, there was little progress in the treatment of cancer and surgery

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[*] samthia@bu.edu; https://orcid.org/0000-0001-5211-266X

remained as the mainstay despite being primitive with many complications, including blood loss, infection, and death. It was not until the 19th and early 20th centuries that significant advances were made in general surgery and cancer surgery as well as from the development of better microscopes enabling the study of cancer tissues to reveal that cancer cells to be markedly different in morphology from the normal surrounding cells from which they originated. In 1838, German pathologist Johannes Müller demonstrated that cancer is made up of cells and not lymph but did not believe it came from normal cells. In 1855, Rudolf Virchow, a student of Johannes Müller, coined his now famous aphorism, omnis cellula e cellula (every cell stems from another cell) and launched the field of cellular pathology and known as the father of cellular pathology. He correlated the clinical course of illness with microscopic pathology findings following surgery and provided the scientific basis for cancer diagnosis and metastatic spread (Tulbah *et al*., 2014).

The American surgeon William Halsted at Johns Hopkins pioneered radical cancer operations in 1894, attempting to outpace tumour growth by more and more extreme removal of tissue, but recurrence indicated that some of the tumour cells have spread. Halsted developed the radical mastectomy, a surgery that removed not only the breast, but also the underlying muscles and nearby lymph nodes, and was the most effective treatment for breast cancer for decades. While radical surgery left many patients disfigured, it also left a legacy. One of Halsted's students at Johns Hopkins Hospital in Baltimore, Hugh Young, under his guidance focused on urological cancers and developed radical prostatectomy, the removal of the prostate gland which cured many men with prostate cancer. Surgery remained as the mainstay of the treatment of solid cancers, until it was joined by radiation and drugs (Tulbah *et al*., 2014).

Radiation kills both healthy as well as cancer cells, but because cancer cells divide faster, it was easy to kill them at higher rate by X-rays. Radiation therapy was pioneered in 1896 by a medical student, Emil Grubbe, without knowing how the treatment worked but now we know it breaks the DNA present in every cell and blocks cell division which subsequently results in cell death. While radiation therapy was believed to be an absolute cure for all forms of cancer, it was soon realized it is only a localized therapy and couldn't be used to treat cancers that have already spread. Additionally, it caused collateral damage to healthy cells and often leading to fresh cancers. Apparently, Grubbe also died of cancers caused by his experiments.

The search for a therapy that in principle can seek out cancer cells wherever in the body even after they have spread was chemotherapy. The era of chemotherapy began with the approval of nitrogen mustard, a poison gas from the First World War that caused destruction of lymphocytes – white blood cells of the soldiers exposed to it was rechristened as mustine, the first licensed chemotherapy agent approved in 1949 by the US Food and Drug Administration to treat lymphoma, a tumour of the lymph system. After this initial success, more chemotherapeutic drugs appeared in rapid succession based on biological insight as well as derived from pure guesswork. In 1947, Sidney Farber at Boston Children's Hospital inspired by the work of British hematologist Lucy Wills's use of Marmite, which is rich in folic acid, a key precursor for DNA synthesis used for treating some forms of anemia, as a potential drug to treat childhood leukemia. When it turned out to be ineffective in treating childhood leukaemia, Sidney Farber decided to try an antifolate drug, aminopterin, the precursor to methotrexate, and found that it caused remissions of leukemia in some patients. This remains as one of the greatest triumphs ever achieved by chemotherapy. Major improvements to cancer therapy were achieved by combining surgery with chemotherapeutic drugs – adjuvant therapy. Because most standard chemotherapies act on all rapidly dividing normal and cancerous cells, improvements were achieved by switching from non-specific brute force to specific targeted interventions that disable or block processes that promote the rapid growth, division and spreading of cancer cells. Prominent successful early targeted therapies include, therapeutic monoclonal antibodies such as trastuzumab (Herceptin) for breast cancer, imatinib (Glivec), a competitive inhibitor of the ATP-binding site of the tyrosine kinase, which prevents substrate phosphorylation, used for treating chronic myeloid leukaemia, and certuximab (Erbitux), a recombinant human/mouse chimeric monoclonal antibody, an inhibitor that binds to the extracellular domain of epidermal-growth-factor receptor (EGFR), used in the treatment of head and neck, lung and colorectal cancers (Arteaga & Baselga, 2012). While classical immunotherapy is considered as the fourth kind of cancer therapy, together with surgery, chemotherapy, and radiotherapy, despite its existence for a long time, it only became very popular due to the recent advances that target the immune checkpoint pathways.

Cancer genome, epigenome and cancer genes

Cancer cells manifest their functional properties due to genetic and epigenetic make ups which continue to evolve. The cancer genome is defined by the 3 billion base pairs of haploid DNA sequence and the sequence variations at a frequency of less than 1% in a population are arbitrarily designated as mutations and those at a higher frequency are referred to as polymorphisms. DNA polymorphisms are primarily assessed by single nucleotide polymorphisms (SNPs) and are the most abundant form of human genetic variation (~10^7) which can be effectively assayed using comprehensive high throughput technologies such as the microarray profiling or simply by using next generation sequencing (NGS) strategies. On the other hand, reversible epigenetic changes consisting of various epigenetic marks are represented by collection of gene specific DNA methylation patterns, unique combinations of post-translational modifications of histones, polycomb group (PcG) proteins, transcription factors and non-coding RNAs/regulatory RNAs [e.g., miRNA (microRNA), lincRNA (large intergenic non-coding RNA) etc.] affecting the mRNA content, define the overall epigenome, which ultimately determines differential gene expression patterns under normal conditions as well as in cancer.

Cancer is a consequence of hyperactivity of growth promoting genes (i.e., oncogenes) which elicit their effect by gain of function and the inactivity of growth constraining genes (i.e., tumour suppressor genes) that manifest their effect due to loss of function. Inherited gene alterations are classified based on their contribution to cancer progression. Alterations in the high penetrance genes (e.g., *APC, p53, BRCA1, BRCA2*) exhibit dominant inheritance of susceptibility and corresponds to two-fold increase in cancer in first-degree relatives and are also known as genes that cause familial cancer syndromes. On the other hand, low penetrance genes (e.g., *CYP, IGF1, UGT1A7, GST*) are represented by subtle sequence variations or polymorphisms and contribute to small to moderate increase in relative risk of cancer and are relatively common in the general population and hence confer higher attributable risk than high penetrance genes.

Cancer phenotypes are driven by gain-of-function alterations as seen with oncogenes such as the *AKT1*, *ALK*, *BRAF*,*CTNNB1*, *DDR2*, *EGFR*, *ERBB2*, *FGFR1*, *IDH1*, *IDH2*, *KRAS*, *MDM2*, *MITF*, *MYC*, *MYCN*, *MYCL1*, *NKX2.1*, *PIK3CA*, *REL* and *SOX2* and/or loss-of-function alterations as frequently observed with specific tumour suppressor genes such as the *APC*, *BMPR1A*, *CDH1*, *CDKN2A*, *NF1*, *NF2*, *MAP2K4*, *MLH1*, *MSH2*, *PIK3R1*, *PTEN*, *RB1*, *SMAD4*, *SMARCB1* and *TP53*, mediated by either genetic or epigenetic changes (Thiagalingam, 2006; Leary *et al*., 2010). The gene alterations that provide a selective advantage during the evolution of a tumour are regarded as the 'drivers' while the alterations that are coincidental in their appearance and do not play a role in the cancer progression are termed the 'passengers' (Haber & Settleman, 2007). While, traditionally, genetic changes were regarded as the drivers and epigenetic alterations as passengers, recent evidence suggest that either type of alteration to be passengers or drivers (Sawan *et al*., 2008). It is also noteworthy that not all mutations in the same gene are drivers as exemplified by *APC* mutations in colorectal cancer (Kinzler & Vogelstein, 1996; Vogelstein *et al*., 2013). Furthermore, some driver genes are more frequently mutated and referred to as the 'mountains', while others, despite their importance, are less frequently mutated and are known as the 'hills', thus shaping the landscape of genetic alterations during cancer progression (Wood *et al*., 2007).

Multi-modular molecular networks (MMMN) of cancer progression

Heterogeneity in the genetic and epigenetic alterations of cancers that correspond to the advancing stages of cancer progression including the terminal metastatic stage, has posed major challenge to accurate diagnosis, prognosis and therapeutic efforts. While there has been significant progress in cataloguing the various genomic and epigenomic alterations with the advent of high throughput technologies, streamlining the available and emerging data into a coherent scheme of events depicting the drivers and the modifiers that culminates in localized and metastatic tumours, requires novel strategies (Thiagalingam, 2006). To address this challenge faced by the cancer research community, I proposed a strategy for the formulation of a detailed framework known as a multi-modular molecular network (MMMN) cancer progression model as a road map to dissect the complexity inherent to cancer (Thiagalingam, 2006; 2015; Figure 1). This model predicts that cancer initiation and progression are mediated by dysregulation/inactivation of a series of interconnected functional sub-network modules. By formulating cancer specific models, one can define functional stages of cancer progression to help design biomarker screening tests for effective diagnosis/prognosis as well as development of target specific cancer therapies.

The MMMN cancer progression model defines a cascade of events encompassing multiple targets within each module, in which one or more alternate target gene(s) could alter the functionality of each of the specific module. This provides a molecular basis for the genomic and epigenomic heterogeneity that is observed during the progression of tumors which exhibit similar pathological characteristics (Figure 1). Furthermore, the absence of consistent alterations in specific gene(s) in sporadic cancers, and in cancers that are primarily induced by environmental effects resulting in neoplastic precursor cells, could be predicted to emerge from inactivation/overactivation of multiple alternate gene targets that act in one or more interconnected axes of events, within a defined sub-network in a module of the global network (Figure 1). The first network module that becomes inactivated leading to the initiation of cancer could be considered as the gatekeeper functional unit (Kinzler & Vogelstein, 1997). The cancer precursor cells harbouring an inactivated gatekeeper module become receptive to additional genomic and epigenomic alterations that occur in interconnected but defined modules of sub-networks through multiple stages, leading to the development of advanced and terminal stages. Therefore, an imaginary frame-work of a succession of modules, the functional inactivation or aberrant hyper-activation of individual network modules occurs in a series of events that advance the tumour from the early to late stages of cancer. Despite the possibility that the overall phenotypic effects elicited by the target tumour or tumour precursor cells could be influenced by the surrounding cells and/or extra-cellular matrix (ECM) components, the genomic and epigenomic and alterations in the resident target cells

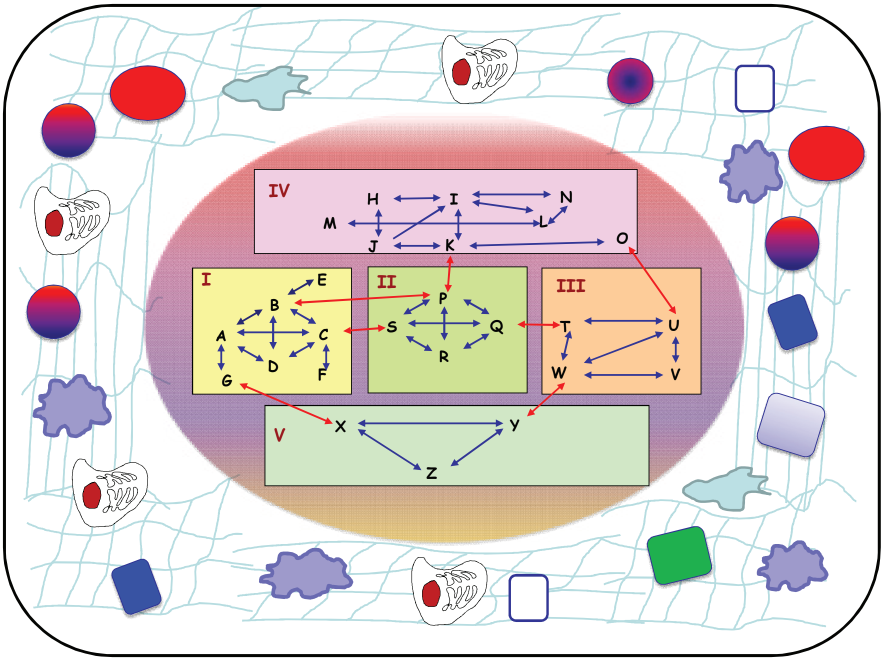

Figure 1: A cascade of aberrant network modules define multi-modular molecular network (MMMN) model for cancer progression

A MMMN cancer progression model predicts that aberrant activations/inactivations of functional modules of networks in a series of steps would be necessary to elicit properties of metastatic cancer. In this model, cancer initiation is mediated by inactivation of the gatekeeper network module (e.g., Module I). We predict that the gatekeeper function is mediated by an interconnecting network of pathways (axes). Dysregulation/inactivation of the gatekeeper module predisposes the cells to become more receptive and susceptible to acquiring additional neolplastic alterations, which occur in a series modular (Modules II, III, IV etc.) inactivations or hyper-activations leading to intermediate and late carcinoma and finally to the metastatic stage. Modules II and III in this model represent the intermediate stages of tumor progression. The terminal module may represent the metastatic stage (module IV). The fact that there could be alternate target genes in any one of the modules of the network could explain why there is often genetic/epigenetic heterogeneity in multi-step cancer progression resulting in similar histologic sub-types of cancer. In this model, the double headed blue and red arrows represent intra- and extra-modular connections, respectively. The alphabetical letters represent specific genes or functional protein-protein and protein-DNA interactions that are nodal points/driver alterations in each network. While the modular organization depicted inside the inner oval represents the alterations within the target tumour cells, the web structures that represent the extracellular matrix and the surrounding stromal cells constitute the tumour microenvironment. Reprinted from the book chapter by Thiagalingam (2015), copyright (2015), with permission from Cambridge University Press.

remains as a pre-requisite for the effects caused by the microenvironment and surrounding stromal and immune cells (Joyce & Pollard, 2009; Taddei *et al*., 2013). It is also noteworthy that overlaps in the functional contributions of the specific gene alterations may be responsible for simultaneous dysregulation of different modules of cancer progression. While any alteration is capable of inactivating/dysregulating a specific sub-network module at any time, its effect will be fully realized to manifest the corresponding cancer stage only when the preceding module(s) have also become inactivated/dysregulated. Thus, the rates at which tumour evolution occurs and the time required for the transition from an early to a later stage of cancer will be dependent upon the preexisting genetic and epigenetic alterations (familial or sporadically acquired) and the tumour microenvironment. This notion is also consistent with an accelerated cancer progression when there is a preexisting inherited alteration that corresponds to a specific module as it has been observed with familial cancers.

The development of cancer cells and their expansion are regulated by immune systems (adaptive and innate) in the tumour microenvironment which recognize the abnormal cells as foreign entities even before they become clinically significant due to external stimuli including cytokines like interferon gamma (IFNγ) and remove them to maintain the normal state. Immune surveillance is mediated by T-cells and other immune cells such as NK-cells that are able to recognize new tumour-associated antigens (TAs) such as the mutated proteins when they are bound to self MHC molecules. There is continuous interaction between cancer cells and the immune system, and the cancer cells may develop escape mechanisms due to low level of immunogenicity or suppression of the immune system by immune checkpoint*s* or due to weakening of the immune system due to spreading of cancer to the bone marrow.

Targeted cancer therapy

After decades of conventional anti-cancer treatment strategies using surgery, radiotherapy and chemotherapy, targeted cancer therapies directed to specific components of cancer cells, such as proteins or genes, that help cancers grow and spread have been developed to minimize the collateral damage to normal cells and to enable the therapies reach the maximum number of abnormal affected cells. Targeted therapies may also be directed at the tumour microenvironment consisting of factors such as cytokines as well as other types of cells that help cancer cells to grow and spread. When samples of tissue (e.g., biopsy) and/or bodily fluids (e.g., blood/serum) derived from a specific patient is subjected to genetic (e.g., mutations/gene amplification) or epigenetic (e.g., DNA methylation/histone modifications) testing and cell surface proteins of the tumour cells/immune cells (e.g., PD-1, PDL-1) and/or molecules in the surrounding microenvironment (e.g., cytokines) are identified, the diagnosis is more accurately made to select the safest and most effective treatment. The two main types of targeted therapies employ either small molecules or monoclonal antibodies. Small molecules are small enough to slip inside cancer cells, block the growth signals or enzymes that make the hormones promoting growth in some cancers such as breast and prostate and destroy them. The small molecule drugs often have their generic name ending in "-ib." (Table 1). On the other hand, monoclonal antibodies which are often referred to as biologics are too big to get into cells and they target the outside of cells or right around them. The generic names of monoclonal antibodies end in "-mab." (Table 2). The third type of targeted therapeutic agents are directed to epigenome coders (Table 3, Thiagalingam, 2020). The FDA in the US and other similar bodies in other countries have approved targeted therapies for more than 15 types of cancers, including those of the breast, prostate, colon, and lung. Targeted therapies may work better than other treatments. Companion diagnostic (CDx) tests and companion biomarkers are often used as the companion to a specific drug and are co-developed for selecting or excluding patient groups for treatment based on biological characteristics that prospectively determine responders and non-responders and predict likely toxicity to the therapy (Duffy & Crown, 2013).

Due to inherent complexity and heterogeneity of tumors (Figure 1), some cancers may not be treatable with just monotherapy and may require a combination of therapies, both traditional and targeted therapies to achieve better patient outcomes (Plana *et al*., 2022). Synergistic interaction is also a common explanation for new combinations. For example, the five-drug R-CHOP regimen cures most patients with diffuse large B-cell lymphoma (DLBCL) (Palmer *et al*., 2018). Erlotinib (EGFR inhibitor) and gemcitabine (antimetabolite chemotherapeutic) are used to treat pancreatic cancer. To target pathways, two strategies can be employed: vertical pathway inhibition, targeting several effectors in the same signalling pathway; or horizontal inhibition, to prevent the overactivation of another pathway in response to targeting one. An example of vertical pathway inhibition is the combination of the BRAF and MEK inhibitors, vemurafenib and cobimetinib for the treatment of advanced BRAF-mutated melanoma. Another example

Table 1: Approved small molecule inhibitors and indications

Drug	Drug target	Biomarker	Indications
Alectinib, Brigatinib, Crizotinib	ALK	ALK fusion	Non-small-lung cell carcinoma
Venetoclax	BCL2	c.CHR17p deletion	Chronic myeloid leukaemia, acute myeloid leukaemia
Bosutinib, Dasatinib, Nilotinib, Ponatinib	BCR–ABL	BCR–ABL	Chronic myeloid leukemia
Imatinib	BCR–ABL, ABL1, KIT, PDGFRA, PDGFRB	BCR–ABL, ABL1, KIT, PDGFR fusion	Chronic myeloid leukemia, acute lymphoblastic leukaemia, myelodysplastic and myeloproliferative disease (with *PDGFR* gene re-arrangements)
Vemurafenib	BRAF V600E	BRAF V600E	Melanoma, Erdheim-Chester disease
Dabrafenib	BRAF, RAF1	BRAF V600E	Melanoma, non-small-lung cell carcinoma, anaplastic thyroid cancer
Ibrutinib	BTK, EGFR, FGR, HER2, RET	c.CHR17p deletion	Chronic lymphocytic leukaemia and small lymphocytic lymphoma
Abemaciclib, Palbociclib, Ribociclib	CDK4 and CDK6	ER positive, PR positive, HER2 negative	Breast cancer
Pexidartinib	CSF1R, KIT, FLT3	Tyrosine kinase	Tenosynovial giant cell tumour (TGCT)
Afatinib, Gefitinib, Osimertinib	EGFR	EGFR T90M, EGFR exon 19 deletion,	Non-small-lung cell carcinoma
Erlotinib	EGFR	EGFR L858R, EGFR exon 19 deletion,	Non-small-lung cell carcinoma, pancreatic cancer
Neratinib	EGFR and HER2	HER2 amplification/ overexpression	Breast cancer
Lapatinib	EGFR, HER2, HER4	HER2 positive, ER Positive, PR Positive	Breast cancer
Enasidenib,	IDH2	IDH2 mutation	Acute myeloid leukaemia
Binimetinib, Cobimetinib, Trametinib	MEK1 and MEK2	BRAF V600E, BRAF V600K	Melanoma, non-small-lung cell carcinoma, anaplastic thyroid cancer
Everolimus	mTOR	ER Positive, PR Positive, HER2 negative	Breast cancer, neuroendocrine tumour, renal cell cancer
Tamoxifen	ER	ER positive	Breast cancer
Olaparib	PARP1 and PARP2	Poly (ADP-Ribose) polymerase, BRCA1/ BRCA2 defects	Breast, ovarian, fallopian tube, and primary peritoneal cancers
Talazoparib	PARP1 and PARP2	Poly (ADP-Ribose) polymerase	Breast cancer
Alpelisib	PI3K	Phosphatidylinositol 3-kinase	Breast cancer
Duvelisib, Idelalisib	PI3K	Phosphatidylinositol 3-kinase	Chronic lymphocytic leukemia and small lymphocytic lymphoma, follicular lymphoma
Letrozole	Aromatase	ER positive, PR positive	Breast cancer
Cabozantinib	c-MET, VEGFR, RET	Tyrosine; CMGC; TKL	Medullary thyroid, hepatocellular carcinoma, and renal cell cancer
Regorafenib	VEGFR	VEGFR	Colorectal cancer, Gastrointestinal stromal tumours, hepatocellular carcinoma
Sorafenib	VEGFR	VEGFR	Hepatocellular carcinoma, renal cell cancer, thyroid cancer (iodine refractory, differentiated)
Sunitinib	VEGFR, PDGFR	VEGFR	Gastrointestinal stromal tumours, pancreatic cancer, neuroendocrine tumour, and renal cell cancer

Table 2: Approved and experimental biologics and indications

Biologic	Target	Mechanism of action	Indications
Bevacizumab (Avastin)	VEGF	Neutralizing antibody	Metastatic colorectal cancer, Non-small-lung cell carcinoma, Glioblastoma, metastatic renal carcinoma
Trastuzumab (Herceptin)	HER2	Receptor blocking antibody	Metastatic breast cancer
Cetuximab (Erbitux)	EGFR	Neutralizing antibody	Colorectal, lung, and head and neck cancers.
Alemetuzumab (Lemtrada)	CD52	Triggers CDC and ADCC on CD52+ immune cells to induce cell death	Chronic lymphocytic leukemia
Ofatumumab (Azerra)	CD20	Triggers CDC and ADCC in CD20 overexpressing B cells causing B cell lysis	Chronic lymphocytic leukemia
Nivolumab	PD-1	Blocks PDL-1 from binding to PD-1 receptor on T-cells	Melanoma, Hodgkin lymphoma, Colorectal cancer, Hepatocellular cancer, Non- small-cell lung cancer, Kidney cancer, Squamous cell carcinoma of the head and neck and Urothelial cancer, Bladder cancer
Pembrolizumab	PD-1	Blocks PDL-1 from binding to PD-1 receptor on T-cells	Gastric cancer, Head and neck cancer, Urothelial bladder cancer
Durvalumab, Atezolizumab	PDL-1	Blocks PDL-1 from binding to PD-1 receptor on T-cells	Urothelial cancer, Non- small-cell lung cancer, Metastatic breast cancer
Avelumab	PDL-1	Blocks PDL-1 from binding to PD-1 receptor on T-cells	Advanced Merkel cell carcinoma, urothelial cancer
Ipilimumab	CTL-A4	Blocks CTL A4 and B7 interaction	Melanoma
Relatlimab	LAG-3	Blocks LAG-3	Recurrent glioblastoma

PD-1 (Programmed death receptor-1) /PD-L1 (Programmed death receptor ligand-1) inhibitors or CTLA-4 (Cytotoxic T-lymphocyte associated antigen 4); LAG-3 (Lymphocyte-activation gene 3)

is anti-angiogenesis agents such as the VEGF inhibitor bevacizumab, in combination with FOLFOX [leucovorin calcium (folinic acid), 5-fluorouracil, and oxaliplatin] or IFL regimen (Irinotecan, 5-fluorouracil, leucovorin) based chemotherapy for treating colorectal cancer. For better benefit, the triplet combination of trastuzumab, pertuzumab, and docetaxel (two HER2 antibodies binding to different epitopes and a microtubule inhibitor) may confer greater antitumor activity for treating advanced stages of NSCLC and breast cancer (Baselga *et al*., 2012).

Cancer immunotherapy as targeted therapy has recently become the preferred treatment over others using chemicals as it is based on improving anti-tumor immune responses with little or no off-target effects. Cancer immunotherapies are designed to prime the immune response to make it more efficient. Different classes of immunotherapies include checkpoint inhibitors, lymphocyte activating cytokines, agonists for co-stimulatory receptors, cancer vaccines, oncolytic viruses, bispecific antibodies and T cell-based adoptive immunotherapy, adoptive cellular therapy (ACT) (Farkona *et al*., 2016; Schmidt, 2017). Cancer immunotherapy has recently received a significant boost from the approval of immune checkpoint inhibitors (ICIs), the anti-cytotoxic T lymphocyte associated protein 4 (CTLA-4) antibody and of anti-programmed cell death protein 1 (PD1) antibodies that cause the inhibition of CTLA-4 and PD-1/PD-L1 axis blockade, respectively, to prolong T cell activity and anti-tumoral effects by blocking co-inhibitory molecules binding to their cognate ligands on the surface of cancer cells (Table 2). For example, ICIs such as nivolumab and pembrolizumab have been demonstrated to mount highly durable response rates with minimal toxicity in patients with advanced melanoma, non–small-cell lung cancer, renal cell carcinoma, and other solid tumours. After failure of a platinum-based chemotherapy, high-PD1 expression in recurrent or metastatic head and neck squamous cell carcinoma (HNSCC) tumour cells or associated immune cells showed better overall survival (OS) and progression-free survival (PFS) outcomes for pembrolizumab. Recent data also suggest that the use of ICIs in combination with the standard chemotherapy regimen improves efficacy of cancer treatment and overall survival (Yan *et al*., 2018; Larroquette *et al*., 2021). For example, PD1/PD-L1 enriched tumours of non-small cell lung cancer (NSCLC) exhibited a potential synergistic effect with treatment combination of pembrolizumab and platinum-based chemotherapy, especially in first-line treatment. Statistically significant improvement in PFS for the pembrolizumab-chemotherapy [nanoparticle albumin-bound paclitaxel (nab-paclitaxel), paclitaxel or carboplatin-gemcitabine] combination compared with placebo-chemotherapy for previously untreated locally recurrent inoperable or metastatic triple-negative breast cancer (TNBC) was observed. However, a limitation of immune checkpoint inhibitors is that they can cause immune-related adverse events (irAEs), usually related to autoimmunity in a dose-independent manner.

Table 3: Epigenome specific drugs and indications

Drug	Target	Mechanism of action	Indications
5-azacytidine (Vidaza) Decitabine (Dacogen)	DNMT	Nucleoside inhibitors	MDS, CML MDS, CTCL, PTCL
Vorinostat (SAHA), Belinostat (Beleodaq)	HDAC	HDAC inhibitors (Hydroxamates)	CTCL, PTCL
Depsipeptide (Romidepsin)		HDAC inhibitor (Cyclic peptide)	CTCL, PTCL
Valporate, Phenyl butyrate		HDAC inhibitor (Fatty acids)	MDS, AML, CLL
Pinometostat (EPZ-5676)	DOT1L	Small molecule inhibitor	MLL-rearranged leukemia
Tazemetostat, GSK126, EPZ6428	EZH2	Small molecule inhibitor	Malignant Rhabdoid Tumors, Lymphoma, Prostate cancer
JQ1, I-BET762, AZD5153, OTX-015, TEN-010, CPI-0610	BET family of BRD proteins	Small molecule competitive inhibitors	MLL-rearranged leukemia, NUT midline carcinoma

AML-acute myeloblastic leukemia; BET- bromodomain (BRD) and extra-terminal family of proteins; CML-chronic myelomonocytic lymphoma; CTCL-cutaneous T-cell lymphoma; DOT1L-disruptor of telomeric silencing-1-like, an exclusive H3K79 methyltransferase; DNMT-DNA methyltransferase, enhancer of zeste homolog 2 (EZH2); HDAC-histone deacetylase; MDS- myelodysplastic syndromes; MLL- mixed lineage leukemia; MM-multiple myeloma; NUT-nuclear protein in testis, p300; PTCL- peripheral T-cell lymphoma

In general, targeted therapies will only work if your tumour continues to harbour the right target and it can also stop working if the target changes as the cancer cells finds a way around the treatment by adapting to parallel or bypass pathways.

Precision cancer therapy

The same cancer type could behave differently from one patient to another, and that there is no one-size-fits-all treatment. Identification of molecular targets at the level of cancer genome, epigenome and the surrounding microenvironment that target the molecular characteristics of an individual's tumour is known as precision cancer therapy (Lee *et al*., 2018; Chen *et al*., 2019). Thus, precision oncology is an approach to diagnosis, treatment and prevention that considers the genes that you're born with (your genetic makeup) and the genes or other markers present within the cancer cells and their microenvironment to personalize therapy. Molecular pathology is playing an instrumental role in driving precision cancer therapy and is increasingly used to guide therapeutic decisions. Some genetic tests are specific to inherited risk, which means they look at your genetic makeup to determine your personal risk of developing cancer in your lifetime. Fundamentally, precision oncology is based upon the idea that tumour biomarkers are predictive of disease phenotype, clinical outcomes, and therapy responses. The emergence of this kind of targeted treatment that uses molecular information to match patients is an exciting moment in the battle against cancer.

The current trend is to identify the fingerprints of the tumours from multiple sources of analysis such as the tumour and cell-free DNA profiling using next-generation sequencing (NGS) technologies, by taking advantage of other methodologies such as in situ hybridization (ISH), reverse transcriptase-polymerase chain reaction (RT-PCR) and immunohistochemistry (IHC) to reveal immunological, proteomic and RNA markers. While these analyses provide previously unavailable details about a patient's cancer that can point the oncologists towards a specific therapy, it also poses a challenge for the analysis of large amount of data from sequencing imaging, screens, and more that are ingested, transformed, and modelled by top open-source machine learning which require the use of advances in computational algorithms and artificial intelligence (AI) (Chakravarty *et al*., 2017; Xu *et al*., 2020; Shao *et al*., 2022). Thus, finding the next big drug candidate might seem merely a step away by "Entering" a key to provide a match with increased medication efficacy and decreased side effects. Of course, the reality is more convoluted, but still promising. All these novel therapeutics are designed to specifically target cancer cells and should cause fewer side effects. In summary, cancer care is among the first medical specialties to apply precision medicine, but for the precision-oncology dream to be fully realized, the therapies must help more people with cancer than the 5–10% who currently benefit.

Conclusions and perspectives

Cancer is one of the leading causes of death, with an estimated 19.3 million new cancer cases (18.1 million excluding nonmelanoma skin cancer) and almost 10.0 million cancer deaths (9.9 million excluding nonmelanoma skin cancer) occurred in 2020 (Sung *et al*., 2021). Cancer incidence is projected to increase to 27.5 million cases by 2040. In recent decades, cancer medicine has evolved from the traditional approaches that targeted any rapidly proliferating cell to an era of precision cancer therapy, in which therapies are targeted to the specific patient's cancer that minimizes side effects. To improve the implementation of precision cancer therapy, this approach should be used early in the course of the disease, and patients should have complete tumour profiling and access to effectively matched targeted therapy. However, due to difficulty in early diagnosis and because of the complexity of tumour biology, clinical trials with combinations of gene-targeted therapy with immune-targeted approaches (e.g., checkpoint blockade, personalized vaccines and/or chimeric antigen receptor T-cells), hormonal therapy, chemotherapy and/or novel agents are continuously being considered and undertaken in several parts of the world. These approaches will greatly benefit from the ability to formulate MMMN cancer progression models for each cancer and the ability to map the affected network(s) and cancer cell surface proteins, corresponding to each person's tumour biology and the dynamic alterations in abnormalities to eliminate minimal residual disease, as well as eradicate majority of the subclones that confer resistance to treatment.

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REVIEW

Vector transport of microplastics bound potentially toxic elements (PTEs) in water systems

M Sewwandi[1], KSD Premarathne[1], H Wijesekara[2], AU Rajapaksha[1,3], S Soysa[4] and M Vithanage[1,3*]
[1] *Ecosphere Resilience Research Centre, Faculty of Applied Sciences, University of Sri Jayewardenepura, Nugegoda 10250, Sri Lanka.*
[2] *Department of Natural Resources, Faculty of Applied Sciences, Sabaragamuwa University, Belihuloya, Sri Lanka.*
[3] *Instrument Center, Faculty of Applied Sciences, University of Sri Jayewardenepura, Nugegoda, Sri Lanka.*
[4] *Department of Physical Sciences and Technology, Faculty of Applied Sciences, Sabaragamuwa University, Belihuloya 70140, Sri Lanka.*

Summary: Microplastics can act as a vector to transport various organic and inorganic contaminants. Hydrophobic and hydrophilic organic contaminants tend to bind to microplastics due to their hydrophobicity and high surface area to volume ratio. Recent studies have focused their attention on evaluating the ability of microplastics to bind potentially toxic elements (PTEs). The co-occurrence of microplastics and PTEs may be facilitated by the ubiquitous presence of both in the environment. The metal adsorption of different microplastics has been investigated under different environmental factors and polymer properties to reveal possible interactions. The environmental factors such as solution pH, dissolved organics, dissolution media, and ionic strength have been studied the most and recognized as factors governing the adsorption of PTEs. Degree of aging and polymer type have been highlighted as the key polymer properties which influence the adsorption of PTEs. However, the effects may differ with different PTEs and environmental conditions. Though sorption capacities and mechanisms have been extensively studied, critical analysis of their behaviour in co-existence with other ions in aqueous media remains unexplored. This review focuses on critically assessing the partition coefficients between different microplastics and water for PTEs in the presence of various factors that influence the metal adsorption. Besides, postulated interactions for the adsorption of PTEs in the presence of dissolved organics, competitive ions, and different pH values are overviewed. Moreover, the associated health risks on biota and humans, when they are exposed to microplastics bound PTEs are also discussed.

Keywords: Ecotoxicity, heavy metals, microplastics, partition coefficient.

INTRODUCTION

The ubiquitous presence and overuse of plastics have become a huge environmental problem up to date, as plastics are recognized as an emerging contaminating pollutant (Alimi *et al*., 2018; Atugoda *et al*., 2020). Due to the uncontrolled usage of plastics stuff, the release of plastics into the environment has distinctly increased. The main pathways to transport plastics into the environment are disposal effluents of wastewater treatment plants, compost, and bio-solids, landfills, treated sewage sludge, and atmospheric deposition (Kershaw, 2016; Kilponen 2016; Rochman, 2018). Any type of plastic nurdles, fragments, or fibres with a diameter ranging from 100 nm to < 5 mm are defined as microplastics (Alimi *et al*., 2018). Based on the formation pathways, microplastics can be categorized into two types as primary microplastics and secondary microplastics (Bradney *et al*., 2019). Synthetically produced microplastics, including plastic nurdles, fibres, and powders for the manufacturing of textiles, plastic products, personal care products, cosmetics, and pharmaceuticals are considered as primary microplastics (Duis & Coors, 2016). Secondary microplastics are formed from the breakdown of the larger plastic debris. As illustrated in Figure 1, microplastics act as leading sources of contaminants harmful to the terrestrial and aquatic environments and life forms.

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[*] Corresponding author (meththika@sjp.ac.lk; https://orcid.org/0000-0003-2923-4065)

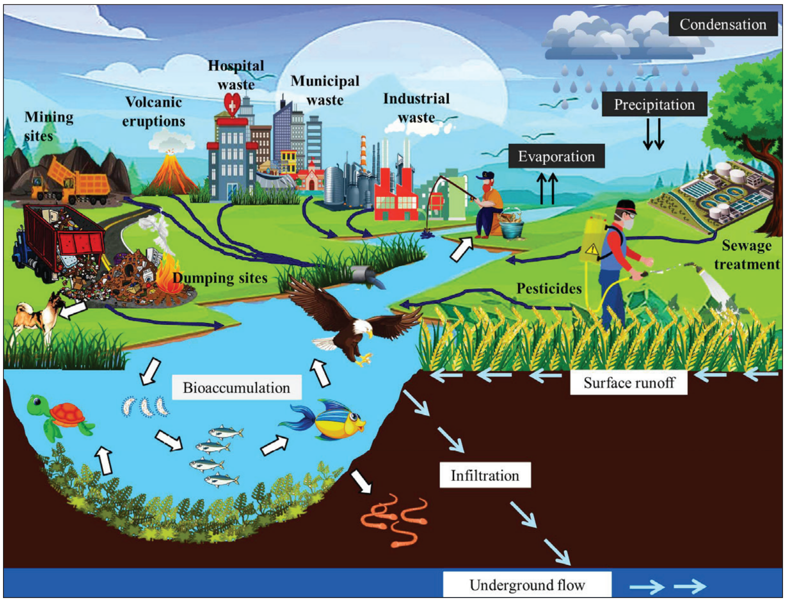

Figure 1: Sources of microplastics and potentially toxic elements (PTEs) and their potential environmental impact during the microplastic cycle in the ecosphere

Due to high hydrophobicity and high surface area to volume ratio of the microplastics, they can greatly facilitate the migration of contaminants through water, acting as a fine vehicle for them (Teuten *et al*., 2007; Rochman, 2018; Tang *et al*., 2020). Microplastics have shown an affinity to both hydrophobic organic pollutants and hydrophilic inorganics such as potentially toxic elements (PTEs). Moreover, plastics can also act as a vector for PTEs influencing their transport through the different environmental systems (Godoy *et al*., 2019; Purwiyanto *et al*., 2020; Fu *et al*., 2021). The surface characteristics of microplastics, such as surface area to volume ratio, porosity, fragment shape, particle size, and reactivity support this vector transportation (Holmes *et al*., 2012; Bakir *et al*., 2014; Brennecke *et al*., 2016). The microplastics bound contaminants may also be an immense threat to the ecosystem due to their combined toxic effects (Banaee *et al*., 2019).

The presence of both PTEs and microplastics in water systems may accelerate the transport of microplastics bound trace metals in water (Guo *et al*., 2020; Aghilinasrollahabadi *et al*., 2021). Further, environmental factors in the adsorption matrix such as solution pH, ionic strength, dissolved organic matter, and temperature of the solution readily facilitate the vector transport of microplastics in water systems (Ahechti *et al*., 2020; Tu *et al*., 2020). Several studies have investigated the implications for the microplastics bound vector transport of PTEs, highlighting its severe effects on living organisms, including humans. Recent studies have demonstrated the ingestion and bioaccumulation of microplastics bound PTEs in the gut and stomach of various species of fish (Oliveira *et al*., 2013; Carbery *et al*., 2018). Moreover, microplastics have been detected in gills and mantle of oysters [4.53 items g^{-1} (wet weight)] while PTEs were found in excessive concentrations (30.5, 4.4, 0.4 and 181.0 μg g^{-1} dry weight of Cr, Cd, Pb and Cu, respectively).

Recently, the interactions and possible mechanisms between PTEs and microplastics have been critically discussed highlighting the effect of environmental factors (Bradney *et al*., 2019; Liu *et al*., 2021). Besides, the combined toxic effects of PTEs and microplastics on organisms have also been reviewed in a few studies (Cao *et al*., 2021; Huang *et al*., 2021). Even though the vector transportation of microplastics bound PTEs has been explored extensively, still their adsorption based on partition coefficients (PCs) and adsorption capacities have not been reviewed critically. As PCs of PTEs for different microplastics are unique for each combination, they need to be comparatively assessed. Therefore, to overcome this knowledge gap, a detailed and critical overview on PCs between microplastics and PTEs existing in water is provided from this review, to tackle issues regarding outstanding microplastics vectors and readily adsorptive PTEs on their surfaces.

The main objectives of this review are to compare and evaluate the existing information on the adsorption of PTEs on microplastics, to have a summarized account on (1) microplastics-PTEs interactions based on (a) pH, dissolved organic matter, ionic strength, and temperature in the solution, and (b) the physicochemical properties of microplastics; (2) partition coefficients between a variety of microplastics and water for different PTEs; (3) the potential environmental impact and health risk of microplastics bound to PTEs on both humans and animals; and (4) major future perspectives to overcome existing research deficits regarding the transportation of microplastics bound to PTEs.

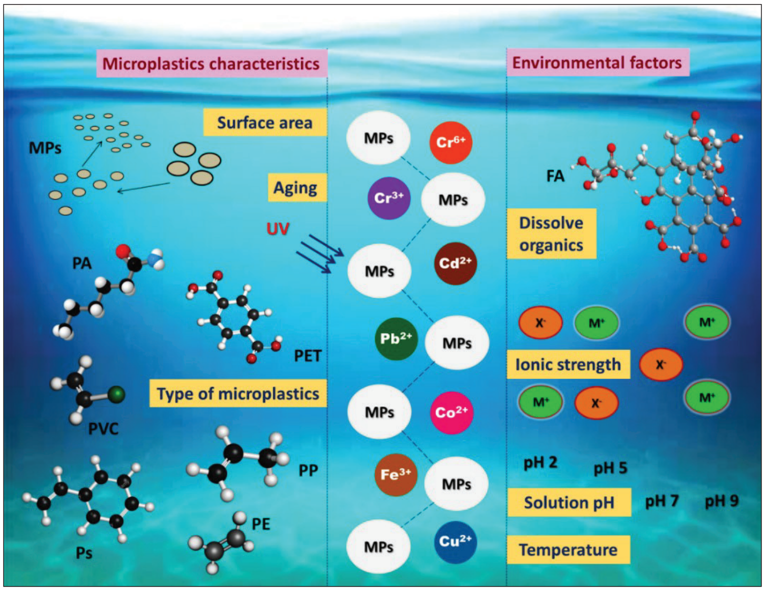

Figure 2: Different environmental factors and characteristics of microplastics influencing the PTEs adsorption on microplastics in water systems

INFLUENCING FACTORS FOR THE MICROPLASTICS BOUND PTES TRANSPORT

Adsorption of PTEs on to microplastics depends upon either the characteristics of microplastics or environmental factors (Figure 2) (Saeedi *et al*., 2018; Mao *et al*., 2020; Tang *et al*., 2020). Several environmental factors such as pH, dissolved organics, ionic strength, and the temperature influence the transport of microplastics bound PTEs, while the properties of microplastics, such as polymer type, surface characteristics, and degree of aging influence the affinity of microplastics for PTEs (Saeedi *et al*., 2018; Richard *et al*., 2019; Mao *et al*., 2020). Those factors may accelerate or interrupt the adsorption process, having positive and negative effects on the binding mechanism.

Environmental factors affecting metal sorption

Effect of pH

Solution pH is an important property that would influence the adsorption of PTEs to microplastics, as it can affect the total chemical reaction, biological toxicity, and equilibrium conditions altering the chemical and biological characteristics of the solution (Marion *et al*., 2011). Additionally, pH can also affect the adsorption of PTEs by influencing metal ion speciation (Yang *et al*., 2019; Dong *et al*., 2020). As an example, Cr may appear at low pH values as $HCrO_4^-$, $CrOH^{2+}$, and Cr^{3+} and at high pH values it appears as CrO_4^{2-}, $Cr(OH)_3$, $Cr(OH)_4^-$ (McNeill *et al*., 2012). Therefore, adsorption of PTEs may drop down due to the formation of non-targeted differently charged metal species that repulse the microplastic surfaces. For instance, at higher pH conditions, Cu is no longer involved in the adsorption process as it appears in the solution as various ionic species ($CuOH^+$, $Cu_2(OH)_2^{2+}$) and precipitated $Cu(OH)_2$ (Yang *et al*., 2019). Similarly, a reduced adsorption was observed due to the speciation of As from H_3AsO_3 to AsO_2^- ions above pH 4 (Sun *et al*., 2010; Dong *et al*., 2020). Consequently, solution pH can greatly effect the adsorption of PTEs by altering their ionic nature. As well, the surface charge of microplastics is highly dependent on the pH of the solution based on its point of zero charge pH (pH_{pzc}) (Tan *et al*., 2008; Xu *et al*., 2008).

Generally, pH_{pzc} of all types of microplastics is less than 7 (Xu *et al*., 2018). When, the solution pH is higher than the pH_{pzc}, the surface of microplastics acquires negatively charged binding sites (Li *et al*., 2019). Consequently, at the environmentally relevant pH values and above, microplastics surfaces are negatively charged, which enhances metal adsorption to the surface through electrostatic interactions. Several studies have evaluated the pH_{pzc} and revealed that sorption is mainly governed by the negatively or positively charged surfaces of microplastics (Atugoda *et al*., 2020; Tan *et al*., 2008). Accordingly, with a slight change of the solution pH, the sorption capacity can be conspicuously influenced by altering the surface charge. However, when the adsorption is not predominately governed by the electrostatic interactions, there is no significant influence from pH.

Effect of temperature in the solution

Depending on the enthalpy change of the sorption mechanism, the adsorption of PTEs on microplastics varies at different temperatures. If the sorption process is endothermic, at higher temperatures microplastics tend to bind PTEs favourably. On the other hand, exothermic sorption processes are interrupted at higher temperatures. Studies on the effect of temperature on the adsorption of PTEs onto different microplastics and documented data are very recent and limited. A few shreds of evidence that highlighted the influence of temperature on adsorptive behaviour can be found in the reviewed pool of literature.

In most studies, adsorption of different PTEs on microplastics has been amplified at higher temperatures, implying that most adsorptions of PTEs are endothermic processes. For instance, Tang *et al*. (2020) showed an increased chemisorption of Pb onto aged nylon microplastics at 313 K, compared to 300 and 288 K. Similarly, polyvinyl chloride (PVC) and polystyrene (PS) achieved their maximum Pb adsorption capacity when the temperature increased from 288 K to 308 K, whereas polyethylene (PE) demolished that trend showing its highest maximum adsorption at 298 K (Lin *et al*., 2021). Comparably, when the temperature increased from 288 K to 308 K, the adsorption capacity of Cu ions onto aged polyethylene terephthalate (PET) microplastics was boosted from 119.4 to 200.5 $\mu g\ g^{-1}$ while that of Zn was raised from 74.8 to 153.6 $\mu g\ g^{-1}$ (Zhang *et al*., 2020). In contrast, As adsorption on PS microplastics was decreased while increasing the temperature, indicating that the adsorption process is favourable at lower temperatures (298 K) (Dong *et al*., 2020). Consequently, it is clear that the temperature of the system can greatly influence the heavy metal sorption onto the microplastics surface. The reported experimental data support prediction of the favourability of the sorption and possible mechanisms, given the thermodynamic nature of the sorption process, to some extent.

Effect of dissolved organic matter (DOM)

Organic matter present in the sorption media is an important factor that can adversely affect the adsorption of PTEs on to the microplastics through complex interactions (Baken *et al*., 2011; Refaey *et al*., 2014; Xu *et al*., 2016; Wijesekara *et al*., 2018). Obviously, the positively charged metal ions have a greater binding affinity to DOM, forming electrostatic interactions or dative bonds with the lone pairs in carbonyl, carboxyl functional groups, and hydroxyl groups existing in fulvic acid (FA) and electron rich aromatic rings and fatty acids moieties contained in humic acid (HA) (Wang *et al*., 2019; Wang *et al*., 2021; Wei *et al*., 2019). The electrostatically or datively bound DOM-metal ion complex facilitates the sorption, retention, and redistribution of metal ions considerably between the solution and the microplastic surface (Gao & Pedersen 2010; Zhao *et al*., 2011). As an example, at higher HA concentrations, adsorption has been increased due the interactions of HA with Pb and Cd ions (Guo *et al*., 2020; Fu *et al*., 2021). Without limiting their binding to PTEs, DOM further interacts with microplastics accommodating more PTEs.

Some microplastics which have π electron containing functional groups (carbonyl, double bonds, aromatic rings) are capable of complexing with HA and FA, forming π- π conjugation and hydrophobic interactions (Guo *et al*., 2015; Chen *et al*., 2018). For instance, PS nano plastics interacted with DOM through π-π conjugation, confirming the interactions between DOM and microplastics (Chen *et al*., 2018). When both DOM and PTEs are treated with microplastics, PTEs have to compete with DOM to cohere to the adsorption sites of the microplastics, as DOM interacts first with the surface of the microplastics through hydrophobic complexation (Refaey *et al*., 2014; Saeedi *et al*., 2018; Wijesekara *et al*., 2018). However, either the combined effect of HA-metal complexation and HA-microplastics complexation or individual effects may positively support the adsorption of PTEs on the surface of microplastics. Apart from positive effects, DOM may also influence the adsorption of PTEs negatively.

At higher DOM concentrations, the complexation of HA with microplastics and HA with PTEs readily inhibits the adsorption of PTEs due to the huge hindrance between the microplastics surface and the metal ions. The complexed PTEs are no longer able to attach onto the microplastic surface (Xu *et al*., 2006; Tan *et al*., 2008). As an example, Pb ions have exhibited a decreased adsorption onto nylon microplastics in the presence of higher FA concentrations, indicating the obstructive effects of DOM on the metal adsorption (Tang *et al*., 2020). Further, the formation of MP-metal-DOM ternary complexes can also be accelerated (Gu & Karthikeyan, 2008; Guo *et al*., 2020). Consequently, DOM in the sorption media can influence the adsorption of PTEs on to microplastics surface negatively or positively. Finally, it can be concluded that the DOM bound to microplastics or DOM in the sorption medium can directly influence the adsorption of PTEs. However, depending on the microplastics type, DOM concentration, and the sequence in which the DOM or PTEs reach the surface of the microplastics, the ways of influence may differ.

Effect of ionic strength

Different types of salt substances except PTEs and DOM, existing in the water system, are one of the most critical influencing factors which determine the extent of the adsorption process. More importantly, the effects of ionic strength on the adsorption can vary depending on factors such as the type of polymer, the distribution coefficient, and speciation of PTEs (Holmes *et al*., 2014; Wang *et al*., 2018; Yu *et al*., 2019). The addition of positively charged ions would alter the electrostatic interactions between PTEs and microplastics by inverting the surface charge and reducing the activity (Mattigod *et al*., 1979; Xu *et al*., 2018). In addition, PTEs have to compete with the positively charged ions for the activated sorption sites (Wang *et al*., 2010; Haas *et al*., 2019; Guo *et al*., 2020). As an example, when cations were added to the solution, Cr^{3+} and Co^{2+} showed reduced adsorption onto PE and PS surfaces due to the competition between the cations for the negatively charged adsorption sites (Godoy *et al*., 2019). The presence of salt ions can disrupt the metal-microplastics interactions by acting as an electrostatic screen between the microplastics surface and the metal ions (Vermöhlen *et al*., 2000; Torres *et al*., 2007). For instance, Cd adsorption on PVC, PE, PS, and PP and Pb adsorption on PVC, PS, and PE have been reduced at higher ionic strengths (Guo *et al*., 2020; Lin *et al*., 2021). Also, in the presence of higher ionic strengths, the number of activated adsorption sites can be reduced as the adsorbent particles start to agglomerate and aggregate (Eren & Afsin 2007; Godoy *et al*., 2020). However, the impact of ionic concentration is not limited to adsorbates and adsorbent; it can further lead to changing the electric potential surrounding the microplastics surface.

Salt ions often commence penetrating an electric double layer on the microplastic surface forming a denser aggregation structure close to the surface (Yin *et al*., 2019). Hence, adsorption of PTEs diminishes at higher ionic strength as the thickness of the electrical double layer begins to decrease, resulting in a reduced electrostatic potential and total free energy (Tang *et al*., 2020). For instance, Tang *et al*. (2020) explained the inhibiting effect of NaCl for Pb adsorption on the nylon microplastics employing the total free energy deduction. However, at higher temperatures, this phenomenon would be changed, because the energy available for the adsorption increases. Moreover, the effects of ionic strength are not same for the adsorption of different PTEs on the same microplastic. As an example, Cd, Co, Ni, and Pb showed significantly reduced adsorption on PE pellets at higher salinity while Cr exhibited enhanced adsorption (Holmes *et al*., 2014). Extrusion effects and salting-out effects in the solution are key factors which can also have an impact on the adsorption of PTEs, for instance, where the extrusion effect is stronger than the salting-out effect in the solution. Consequently, it can be noted that ionic species in the sorption matrix have an effect on the adsorption of PTEs in different ways, with either positive, negative, or zero effects depending on the type of microplastics.

Effect of the properties of microplastics

Physical properties

Surface properties of the microplastics such as surface area to volume ratio, porosity, fragment shape, and particle size can play a vital role in influencing the metal adsorption on to the microplastics surface (Bakir *et al*., 2014; Holmes *et al*., 2014; Brennecke *et al*., 2016). Depending on the shape of microplastics, virgin PS beads have exhibited a decreased adsorption due to their smaller surface area tovolume ratio than aged PVC fragments having an irregular shape with a higher surface area (Brennecke *et al*., 2016). In addition, the adsorption of As was decreased when increasing the particle size of PS microplastics indicating that smaller particle sizes enhance As adsorption (Dong *et al*., 2020). According to the specific surface area of the PE, PP, PS, and PVC microplastics, the sorption rate of Cd ions onto their surfaces was increased in sequence PE < PP < PS < PVC (Guo *et al*., 2018; 2020). By contrast, surface properties of microplastics are not always the main factor for capturing Pb. As an example, though the order of surface area per unit of mass was PS > PP > PE > PVC > PET, the adsorption performances were changed without any order indicating that the adsorption was probably not depended on all surface chemical and physical properties (Godoy *et al*., 2019). Therefore, the sorption behaviour of metals to microplastics would be expected to differ with the change of surface area, shape, and hydrophobicity of each type of microplastics (Teuten *et al*., 2007; Karapanagioti & Klontza, 2008).

Degree of aging

During the aging process, the surface of microplastics begins to change the surface uniformity, becoming rough and forming pores. Further, aging leads to change in particle size and degree of folding of microplastics (Lang *et al*., 2020; Luo *et al*., 2020). Therefore, these possible surface changes increase the surface area of microplastics promoting the vector transport of metal pollutants (Paul-Pont *et al*., 2016; Zhang *et al*., 2020). Aged PVC fragments showed comparatively higher Cu adsorption capacities than virgin PS beads (Brennecke *et al*., 2016). Moreover, the generated wrinkles and cracks during the aging process led to increasing the number of activated sites for metal adsorption, and hence accelerate the adsorption through a pore filling mechanism (Lang *et al*., 2020; Mao *et al*., 2020). Aging time and aging environment also influence or enhance metal adsorption (Luo *et al*., 2020). Illustratively, degree of aging of PS microplastics in different environments were UV air > UV seawater > UV pure water (Mao *et al*., 2020). Aging also eases the adsorption by oxidizing the functional groups in the microplastics, forming carbonyl and carboxylic groups (Fotopoulou & Karapanagioti, 2012; Liu *et al*., 2019; Mao *et al*., 2020). Further, processes such as photo-oxidative weathering, accumulation of biofilms, and chemical hydrogenous precipitates can also influence the metal adsorption on microplastics (Morét-Ferguson *et al*., 2010; Holmes *et al*., 2012). Additionally, newly formed polar moieties like carbonyl and carboxylic groups containing lone pairs in aged microplastics preferentially attract metal ions through electrostatic interactions or dative bonds (Cao *et al*., 2021). Accordingly, it is clear that aging promotes the adsorption of PTEs by changing the physical and chemical characteristics of microplastics surface.

Type of microplastics

The metal sorption capacity may be influenced by the type of microplastics. The specific functional groups in microplastics, such as, chlorine, amide, hydroxyl, carboxyl, and aromatic enhance the metal adsorption through electrostatic and hydrophobic interactions by increasing the polarity of the polymer surface. Moreover, the affinity of positively charged metal ions to polar functional group makes the adsorption easier through electrostatic or dative bonds (Cao *et al*., 2021; Liu *et al*., 2021). Interestingly, most common plastics such as PS, PA, and PET have at least one π-electron rich or polar region which is able to attract metal ions. The hydroxyl and carboxyl groups introduced to the polymer structure of PS microplastics during the ball milling process have collectively provided positive electrostatic potential for As adsorption (Dong *et al*., 2020). Furthermore, for the Pb adsorption, PS microplastics have exhibited the maximum sorption rate, while PE and PVC showed a smaller sorption rate (Lin *et al*., 2021). Due to the presence of a more polar chlorine group in PVC chain, it has adsorbed a higher amount of Cu than PS microplastics (Brennecke *et al*., 2016). Therefore, it can be concluded that the type of microplastics can crucially affect the adsorption of PTEs.

ADSORPTION ABILITY OF MICROPLASTICS TO PTES

Partition coefficient (PC) value is a more important parameter to understand the real performance of adsorbent in binding to the contaminants, than the adsorption capacity and the removal efficiency. The equilibrium PC for a given system under constant pressure, temperature, and matrix composition such as pH, ionic strength, and DOM, is independent of both adsorbate and adsorbent concentrations (Zhao & Hou, 2012). Hence, the bias in both adsorption capacity and removal efficiency can be reduced by using PC values to discuss the potential of adsorbents (Ramanayaka *et al*., 2019; Vikrant & Kim 2019; Ashiq *et al*., 2021). Therefore, PC values of the reviewed microplastics are listed in Table1 to assess the true adsorption ability of microplastics with respect to PTEs. Higher PC values indicate the magnified adsorption potential of microplastics.

Numerous studies have reported that Pb and Cr have a higher affinity to adsorb onto pristine or aged microplastics such as PP, PS, PE, PVC, PET, and nylon (Tan *et al*., 2008; Li *et al*., 2020; Tang *et al*., 2020). The high adsorption ability of all microplastics varieties is likely supported by the large amount of dissolved organics in the adsorption matrix to bind more Pb (Godoy *et al*., 2019). When the sorption media was sea water, aged PE pellets have showed remarkable adsorption performance for both Pb and Cr, compared to river water, highlighting stronger adsorption than virgin PE pellets (Holmes *et al*., 2012; 2014; Lin *et al*., 2021). The enhanced adsorption may be

Table 1: Adsorption capacities and partition coefficients of reviewed microplastics to different potentially toxic elements (PTEs)

Type of microplastic	PTE	Adsorption capacity (μg g^{-1})		Partition coefficient (L g^{-1})		Sorption medium	Reference
		Pristine	Aged	Pristine	Aged		
PE Pellets	Cr	29.70×10^{-2}	44.10×10^{-2}	5.50×10^{-5}	22.10×10^{-2}	Sea water	(Holmes *et al*., 2012)
	Co	1.80×10^{-2}	3.80×10^{-2}	1.00×10^{-5}	4.03×10^{-3}		
	Ni	0.80×10^{-2}	7.00×10^{-2}	1.40×10^{-5}	8.87×10^{-3}		
	Cu	26.10×10^{-2}	-	3.30×10^{-5}	4.52×10^{-2}		
	Cd	0.04×10^{-2}	1.00×10^{-2}	2.00×10^{-9}	7.94×10^{-3}		
	Pb	-	71.60×10^{-2}	2.00×10^{-8}	0.15		
PE pellets	Cd	1.00×10^{-2}	0.25	2.01×10^{-3}	5.23×10^{-2}	River water	(Holmes *et al*., 2014)
	Co	6.89×10^{-2}	7.96×10^{-2}	$1.40x\ 10^{-2}$	1.62×10^{-2}		
	Cr	-	4.21×10^{-2}	-	1.90×10^{-2}		
	Cu	10.04×10^{-2}	-	2.01×10^{-2}	-		
	Ni	1.66×10^{-2}	15.14×10^{-2}	3.32×10^{-3}	3.46×10^{-2}		
	Pb	19.10×10^{-2}	273.50×10^{-2}	3.97×10^{-2}	1.21		
PE pellets	Cd	0.43×10^{-4}	1.00×10^{-2}	8.60×10^{-6}	2.04×10^{-3}	Sea water	
	Co	1.76×10^{-2}	4.21×10^{-2}	3.54×10^{-3}	8.49×10^{-3}		
	Cr	29.74×10^{-2}	44.09×10^{-2}	6.32×10^{-2}	9.67×10^{-2}		
	Cu	26.12×10^{-2}	-	5.51×10^{-2}	-		
	Ni	0.76×10^{-2}	7.34×10^{-2}	1.52×10^{-3}	1.49×10^{-2}		
	Pb	-	68.38×10^{-2}	-	0.16		
PE pellets	Ag	1.28×10^{-2}	1.07	1.81×10^{-3}	9.63×10^{-2}	River water	(Turner & Holmes, 2015)
	Cd	1.01×10^{-2}	0.25	3.47×10^{-4}	-		
	Co	6.92×10^{-2}	0.08	3.04×10^{-4}	-		
	Cr	-	0.09	1.01×10^{-3}	4.60×10^{-3}		
	Cu	10.00×10^{-2}	-	3.62×10^{-3}	6.10×10^{-2}		
	Hg	17.00×10^{-2}	2.78	5.84×10^{-3}	3.18×10^{-2}		
	Ni	1.66×10^{-2}	0.15	4.91×10^{-4}	-		
	Pb	19.10×10^{-2}	2.74	5.80×10^{-3}	0.22		
	Zn	-	-	-	-		
LDPE	Cr	1.70	-	1.73	-	Sea water	(Farhan *et al*., 2018)
Mixture of PP, PE, PES, PVC, and nylon	Pb	-	0.47	-	-	River water	(Purwiyanto *et al*., 2020)
	Cu		0.09				
PE	Al	6.20	-	-	-	After suspended in harbor sea water for 8 weeks	(Ashton *et al*., 2010)
	Fe	17.98					
	Mn	2.61					
	Cu	0.28					
	Pb	1.72					
	Zn	0.25					
	Ag	2.47×10^{-2}					

Continued -

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Type of microplastic	PTE	Adsorption capacity (μg g^{-1})		Partition coefficient (L g^{-1})		Sorption medium	Reference
		Pristine	Aged	Pristine	Aged		
PE	Cr	3340.00	-	71.67×10^{-2}	-	Distilled water	(Godoy *et al*., 2019)
		2560.00		47.06×10^{-2}		Sea water	
		7900.00		79		Urban water	
		6670.00		5.02		Irrigation water	
	Pb	1770.00	-	28.41×10^{-2}	-	Distilled water	
		3280.00		69.49×10^{-2}		Sea water	
		7630.00		20.62		Urban water	
		5690.00		2.46		Irrigation water	
PET	Pb	2310.00	-	40.60×10^{-2}	-	Distilled water	
		3730.00		87.35×10^{-2}		Sea water	
		7520.00		15.67		Urban water	
		5260.00		1.92		Irrigation water	
	Cu	1590.00	-	24.80×10^{-2}	-	Distilled water	
		2870.00		55.95×10^{-2}		Sea water	
		6240.00		3.55		Urban water	
		995.00		14.20×10^{-2}		Irrigation water	
PP	Pb	2290.00	-	40.11×10^{-2}	-	Distilled water	
		3150.00		64.95×10^{-2}		Sea water	
		7660.00		22.53		Urban water	
		5170.00		1.83		Irrigation water	
	Co	535.00	-	7.17×10^{-2}	-	Distilled water	
		400.00		5.26×10^{-2}		Sea water	
		239.00		3.08×10^{-2}		Urban water	
		395.00		5.19×10^{-2}		Irrigation water	
PS	Pb	2390.00	-	42.60×10^{-2}	-	Distilled water	
		3290.00		69.85×10^{-2}		Sea water	
		7400.00		12.33		Urban water	
		5640.00		2.39		Irrigation water	
	Cr	1730.00	-	27.59×10^{-2}	-	Distilled water	
		6140.00		3.30		Sea water	
		7680.00		24.00		Urban water	
		7640.00		21.22		Irrigation water	
PVC	Pb	1100.00	-	0.16	-	Distilled water	
		3450.00		0.76		Sea water	
		7600.00		19.00		Urban water	
		5080.00		1.74		Irrigation water	
Mixture of PE and PP films and fragments (<5mm, mainly 2–3mm)	Ni	-	0.50 – 2.40	-	-	River sediments	(Wang *et al*., 2017)
	Cd		2.10 – 17.60				
	Pb		38.20 – 131.10				
	Cu		80.90 – 500.60				
	Zn		2414.00 – 14815.00				
	Ti		13617.00 – 38823.70				
Orange, filamentous PE	Cd	-	3.94	-	-	Beach sand	(Turner, 2017)
	Cr		3.28				
	Pb		8.40				

governed through the electrostatic interaction between metal ions and PP and PE microplastics. Reviewed data clearly indicate that when the adsorption matrix rich in ions and DOM, both Cr and Pb ions have higher affinity to microplastics which contain more polar moieties.

Copper was excellently adsorbed onto virgin PET where urban water was the adsorption medium (Godoy *et al*., 2019). At high temperatures, pristine PET can also act as a leading adsorbent for Cu (Zhang *et al*., 2020). Besides, PS, PE (either as the virgin or aged form), and polymethylmethacrylate (PMMA) also have exhibited their ability to act as a carrier for Cu ions (Yang *et al*., 2019; Mao *et al*., 2020). In conclusion, it can be clearly identified that, at high temperatures, Cu has a high affinity for PMMA, PS, PVC and PET microplastics in the presence of surfactants and DOM rich sorption medium.

Adsorption of Zn is quite different to other PTEs, as a variety of microplastics, such as PS, PE, PET, PVC, and a mixture of PE and PP microplastic, has exhibited their adsorption potential towards Zn (Godoy *et al*., 2019). Contrastingly, both virgin and aged PE pellets have shown zero adsorption of Zn in the presence of river water (Turner & Holmes, 2015). However, pristine PS microplastics have showed a higher adsorption ability for Zn than for Cu, while aged PVC microplastics showed nearly the same performance for both Cu and Zn in the absence of antifouling paint treatment, in seawater (Brennecke *et al*., 2016). Nevertheless, when compared to the other PTEs, microplastics have demonstrated a lesser adsorption of Zn regardless of the sorption medium.

Virgin PS microplastics have showed the highest adsorption ability for Co through a significant enhanced adsorption in Milli-Q water than other types of water (Godoy *et al*., 2019). Thus, adsorption potential of PS microplastics for Co was again higher than that of both virgin and aged PE pellets where seawater is the adsorption medium (Holmes *et al*., 2012; Godoy *et al*., 2019). However, with the limited data for Co adsorption the trend of its adsorption cannot be predicted precisely. Nevertheless, according to the available data, PS microplastics have showed outstanding adsorption ability for Co where the sorption matrix was not crowded with competing ions and organic matter.

Very little reported data on PC based adsorption performance of microplastics to bind Cd are found in the literature to date. Instead of PC values, most studies have investigated only the adsorption capacities of different microplastics (Turner 2017; Wang *et al*., 2017). Nevertheless, both aged and pristine PE pellets have showed low PC values for Cd where the sorption medium was seawater and river water (Holmes *et al*., 2014). The study by Turner and Holmes (2015) reported very similar behaviour to that of Holmes *et al*. (2014). However, to have a clear elucidation about PC values for Cd adsorption on microplastics, the reported data is not sufficient.

The adsorption of Sr, As and Ni to different microplastics has not been studied much. However, pristine PE microplastics have shown a strongly enhanced adsorption of Sr with a 3.92 L g^{-1} PC value, compared to that for the PET microplastics. In addition, PS microplastics adsorbed As ions resulting in a 0.023 L g^{-1} PC value (Dong *et al*., 2020). Presumably, a few studies have conducted adsorption experiments to elucidate the capability of Ni to adsorb on to the aged PE and PS microplastics compared to pristine ones. Nevertheless, microplastics bound transportation of some PTEs such as Hg, Ag, Al, Ti, and Mn, which are likely to be harmful to the environment, remain relatively unexplored. Lastly, assessment of the available PC data in the literature strongly contributed to a discernment of the adsorption of PTEs on different microplastics to some extent, elucidating the role of microplastics as sinks or sources of PTEs.

POTENTIAL ENVIRONMENTAL IMPACT

Due to the tiny particle sizes and highly availability in the environment, microplastics can be readily ingested by organisms mainly through food webs and water. For instance, microplastics can be easily mistaken for plankton (Egbeocha *et al*., 2018), due to their small size and shape; hence these can be accidentally eaten by a range of small invertebrates and vertebrates that live in natural ecosystems (Mathalon & Hill, 2014; Huerta Lwanga *et al*., 2016). Ingestion of microplastics has directly damaged organs in the digestive tract and reproductive system, leading to a decline or decrease of growth, feeding rate, nonlethal morphology and reproductive output of marine organisms (Murphy & Quinn, 2017; Curren *et al*., 2020). Besides, when microalgae adsorb plastic particles their photosynthetic processes may drop, leading to an increase in reactive oxygen species (ROS) and having an adverse impact on biologically important molecules, including DNA, protein, and lipid, by interacting with other molecules (Liu *et al*., 2019; Prokić *et al*., 2019). Even so, microplastics may remain in the gut for a longer time as a result of the formation of hetero aggregates, leading to an enhanced bio-accumulation in higher trophic levels (Carbery *et al*., 2018; Egbeocha *et al*., 2018). As an example, different microplastics such as PS, PE, and PP, have been discovered in commercial fish (Cho *et al*., 2019; Robin *et al*., 2019). However, apart from their individual effects, microplastics cause more harmful impacts on organisms during their vector transport, with combined toxic effects (Liu *et al*., 2019).

High levels of PTEs (As, Cd, Cr, Cu, Ni, Pb, and Zn) and microplastics (PE, PET) can be found in both marine and freshwater animals (Murphy & Quinn, 2017; Zhu *et al*., 2020; Sarkar *et al*., 2021). However, the combined toxic effects may vary with the type of plastics, selected PTE, and test species (Liu *et al*., 2021). The ingestion of microplastic bound PTEs have higher potentially harmful effects upon the feeding behaviour, growth, and reproduction of marine organism (Anderson *et al*., 2016; Botterell *et al*., 2019). For instance, when Cd and microplastics were applied to carp, their blood biochemical and immunological indicators were significantly changed (Banaee *et al*., 2019). Also, microplastics most probably concentrate in the digestive system, influencing food intake and oxygen uptake, and later with more effect upon the absorption of nutrients (Zhu *et al*., 2020). Moreover, desorption of microplastics bound PTEs in gut fluids is facilitated by various gut fluid components (Hodson *et al*., 2017). In addition, the presence of microplastics in fish accelerate their intake of heavy metals, which induce chronic health issues in humans through food chains (Liu *et al*., 2021). The impacts on humans depend upon dose, level of exposure, age, sex, and genetics (Godoy *et al*., 2020). Accordingly, long term consumption of microplastics bound PTEs may lead to prolonged lethal effects in both human and biota.

FUTURE PERSPECTIVES

Although important and enormous findings are available to describe the sorption behaviour of metal contaminants with microplastics, considerable data gaps have arisen when interpreting their sorption. Hence, the following unexplored issue should be studied extensively. Though theoretically it is considered that the equilibrium PC is a system constant, independent of adsorbate and adsorbent concentrations, several studies have experienced that experimentally measured PC values of a particular system decreased with increasing adsorbent concentration (Hatje *et al*., 2003; Kim *et al*., 2003). Therefore, the PC itself has several limitations for evaluating the adsorption performance. Accordingly, future studies are needed to focus on new parameters, to explain adsorption capacities precisely. Since non-polar and non-degradable microplastics have been widely used in previous studies, future work should be mostly focused to determine the heavy metal adsorption affinity of polar and degradable microplastics with regard to their functional groups. The precise role of aged microplastics and their effect on the adsorption of PTEs deserves to be further studied in order to identify the exact mechanisms, including the formation of new binding sites, use of weathering layers as a precipitation nucleus, etc., that account for efficient metal retention on microplastics. Most studies on the adsorption of PTEs were performed with the use of laboratory-aged or field-extracted weathered microplastics under simulated laboratory conditions. Hence, future investigations should be urgently directed towards experiments under environmentally realistic conditions. Also, future investigations ought to emphasize the solid-liquid interactions which may support the adsorption of PTEs.

Researchers should pay attention to evaluate the role of a mixture of microplastics in transporting and retaining a broader range of PTEs. Thus, it is necessary to study how the competing ions in the water systems act on the adsorption of PTEs. In addition, sorption studies under microbial exposure merits future study. Besides, enthalpy, entropy, and free energy changes of the adsorption processes should be examined to understand the thermodynamics. Further, particular attention must be paid to study the desorption of heavy metals adsorbed on microplastics in fragile ecosystems. Additional information is needed to determine the burden of plastics-bound heavy metals on wildlife, their migration to different organisms, and possible conflicting effects. Also, a realistic risk assessment is urgently required to address the possible risks associated with metal-bound microplastics to the whole ecosystem. Moreover, an exploration of the impact of heavy metal release from microplastics under different gut environments is needed.

Conflict of interest

The authors declare that they have no know competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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