Journal *of* Arts Science *and* Technology

Vol. 15, No. 2 – Special Issue September 2023

SPECIAL ISSUE

Proceedings of the Inaugural Frontiers of Research in Caribbean Science and Technology (FORECAST) 2022 Conference

August 10–12, 2022

"Science & Technology: A D.R.I.V.E.R. of Transformation"

A PUBLICATION OF THE UNIVERSITY OF TECHNOLOGY, JAMAICA

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Journal of Arts Science and Technology (JAST)
Volume 15, No. 2 (Special Issue)

Published by
The University of Technology, Jamaica
237 Old Hope Road
Kingston 6, Jamaica, West Indies
Tel: 876-927-1680-8
Website: https://www.utech.edu.jm/academics/sgsre/publications-1/publications
e-mail: jasteditor@utech.edu.jm

ISSN: 0799-1681

Cover and journal design by Robert Harris
(e-mail: roberth@cwjamaica.com)
Set in Minion Pro 11.5/15 x 30

Editorial Board

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Note from the Editor-in-Chief

The Journal of Arts Science and Technology (JAST) is the flagship journal of the University of Technology, Jamaica and its publication is in keeping with one of the objects of the University of Technology Act, to "preserve, advance, and disseminate knowledge through teaching, scholarship and research . . . and to make available the results of such . . . to promote wisdom and understanding".

This is a Special Issue of *JAST* comprising the **Proceedings of the Inaugural Frontiers of Research in Caribbean Science and Technology (FORECAST) Conference** that was held online from 10–12 August 2022.

The Editorial Board and Management Committee of *JAST* are pleased to partner with the organizers of the conference to publish this Special Issue.

Paul W. Ivey, PhD
Editor-in-Chief

Guest Editors' Editorial

The Frontiers of Research in Caribbean Science and Technology (FORECAST) Conference was the first of its kind; a multi-institutional event orchestrated and hosted by the science and technology faculties of The University of the West Indies Mona, Cave Hill and St. Augustine campuses, and the Faculty of Science and Sport at the University of Technology, Jamaica.

The conference was held from 10–12 August 2022, as an entirely online event which was another first for the participating organizing institutions. The conference that was held under the theme "Science and Technology: a D.R.I.V.E.R. of Transformation" facilitated the timely gathering and meeting of the minds as the region began to emerge from the difficult period of the COVID19 pandemic restrictions. The conference provided many opportunities for networking, the discussion of science, hypotheses, and experiments.

This Special Issue of the *Journal of Arts, Science and Technology* contains a selection of full papers that were developed from presentations made at the conference and subsequently peer-reviewed by experts across the Caribbean and other territories. This selection includes papers that fall under one or more of the conference's general areas of interest: development, resilience, innovation, vision, entrepreneurship, and renewal. The acceptance rate for the papers included in this special issue was approximately 17%.

This carefully curated collection of research being conducted in the Caribbean showcases the prowess of Caribbean scientists and the important and relevant work that they have undertaken. The papers describe research, studies, experiments, and results in both the Caribbean's blue and green economies, and technology fields.

In the first paper titled '**Science, Technology and Innovation (STI) Policy: A necessary artefact for the Small Island Developing States (SIDs) of the Caribbean'**, the author presents perspectives on the social and economic transformation that is possible through the careful formulation and execution of robust science and technology policy, and postulates that the resulting innovations could then spur sustainable social transformations and long-term economic growth within the region.

'Bioprospecting and Biopiracy in the Caribbean: Challenges and Opportunities for the Region', is the second paper and it draws our attention to the threats posed to our biodiversity, including biopiracy. The authors report on bioprospecting cases that have, or have not, benefited the region and biopiracy cases which are also now providing benefits to the Caribbean.

The authors of the third paper **'Preliminary Evaluation of Hygienic Practices of Street Food Vendors in Barbados'** evaluated the hygienc practices of street food vendors in Barbados with a focus on the prevention of food contamination. The research team presents data acquired through the use of a survey that sought to assess the microbiological quality of a small selection of popular Barbadian street food.

In the fourth paper **'Assessment of Small-Scale Cacao (Theobtoma cacao L.) Fermentation Method for Niche Marketing'**, the authors investigate the importance of fermentation of cacao and its importance in the expression of genetic flavour. This and other aspects of processing are of particular importance as the paper also highlights the burgeoning of small gourmet chocolate boutiques worldwide and the increased interest in niche marketing of cocoa microlots.

The fifth paper, **'The Impact of Land Management on Soil Health in Jamaica: A Case Study of the Millibank Farming Region'** presents the findings of a case study that examined numerous abiotic factors as proxies to decipher the impact of land management on long-term soil health in rural farming communities of the Upper Rio Grande Valley in Jamaica.

In the sixth paper, the authors of **'Temperature and Chemical Induced Conformational Changes in Purified PEPC'** show how temperature fluctuations cause conformational changes on the secondary and tertiary structure of the phosphoenolpyruvate carboxylase (PEPC) protein purified from the leaves of the Amaranthus hypochondriacus.

The seventh paper '**Host Preference, Impact of Host Transfer and Insecticide Susceptability among Aphis gossypii Group (Order: Hemiptera) in Jamaica'**, concerns one of the world's most polyphagous agricultural pests, the Aphis gossypii, and its developed resistance to many commonly used insecticides. In their study, the authors examined the level of insecticide susceptibility amongst these aphids in Jamaica while exploring fecundity and colony growth as a measure of host preference and host transfer success.

The author of the eight paper, **'A Baseline Assessment of Lionfish (Pterois spp.) Population Dynamics, Distribution and Diet within the Montego Bay Marine Park, Jamaica in 2018'**, describe the venomous marine fish that have invaded the waters of the Atlantic Ocean and the deleterious effects that they pose to an environment's overall health and functioning by the consumption of keystone species such as the parrotfish. The author presents data collected that projects the general distribution, size, maturity and diet of the Lionfish within the Montego Bay Marine Park.

The authors of paper nine **'Emerging Trends in Data Utilising Longitudinal Analysis'**, present the results of a study that aimed to analyse the trends in data using longitudinal analysis, with particular focus on divorce rates versus success in school, and sustainable development goals (SDG) versus incidence of multidrug resistant tuberculosis (MDR-TB).

Paper ten is **'Simulation Driven Investigation of the Effect of SnS, ZnO and Mo(S,Se)2 Layers on a GaAs/AlGaAs Heterojunction Solar Cell** and investigated the current-voltage, current-wavelength and electric field penetration capabilities in a simulation of three (3) layers of tin sulphide, Molybdenum and zinc oxide on a wafer of gallium arsenide and aluminium gallium arsenide. This simulation is of particular importance in its consideration for its use in solar energy cells.

The availability of a daily, ground-measured, global, horizontal irradiation dataset provided by the Trinidad and Tobago Meteorological Service, enabled the authors of paper eleven, to undertake an **'Evaluation of the Inter-Annual Variability of Site-Corrected Daily Global Horizontal Solar Irradiation in Trinidad and Tobago'** to show that short periods of measurements (1 year) could potentially be used to explain the long-term characteristics of the solar resource in Trinidad and Tobago.

And finally, paper twelve, '**To be or not to be: Inducers, Inhibitors and Implications for Transport Decarbonisation in a Small Island'** presents an investigation into whether sector experts consider the sustainable decarbonisation of the Barbados transport sector by 2030 to be viable and includes their supporting reasons and potential policy implications.

We express deepest gratitude to all who were involved in making FORECAST 2022 such a resounding success. We are particularly thankful to our sponsors for their financial support – without our sponsors, FORECAST 2022 would not have been possible: Barbados Investment and Development Corporation; The UWI Mona Research and Publication, and Graduate Awards; The UWI St. Augustine; The UWI Cave Hill; University of Technology, Jamaica; InterAmerican Development Bank; Oran Family Trust; Huawei; UNICOMER; Payce Digital; Caribbean LED; The International Software Architecture Qualification Board (iSAQB); Barbados Port Inc.; Gildan; Lime Grove Lifestyle Centre; Ansa Merchant Bank;

Jamaica National; National Water Commission; CEMEX; and Jamaica Energy Partners.

We also thank the Editorial Board and Management Committee of *JAST* for readily agreeing to publish this Special Issue.

We are confident that you find this special issue of *JAST* engaging and informative. We look forward to the next staging of FORECAST in 2024 and the continued growth, development, and international contributions to science and technology from our Caribbean scholars.

Curtis Busby-Earle, PhD
Andrew Lamm, PhD
Guest Editors

Journal of Arts, Science and Technology

Science, Technology, and Innovation (STI) Policy

A Necessary Artefact for the Small Island Developing States (SIDs) of the Caribbean

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Abstract

Economic development theorists have established the theoretical constructs of scientific and technological developments as foundations to innovative solutions in the form of products, processes and services to long-term socio-economic development and prosperity to high-end economies around the world. It is the pervasiveness of these innovations within markets which then spurs a sustainable social transformation and long-term economic growth within nations. The perspectives presented in this paper are that, within the context of small island developing states (SIDs), social and economic transformation is attainable through the formulation and execution of robust science and technology (S&T) policy. However, this author is positing that a Science and Technology policy must include the 'I' – Innovation – to become a Science, Technology and Inniovation (STI) policy which then establishes the foundation for the formulation of appropriate artefacts – legislative and regulatory as well as systems of innovation namely sectoral systems of innovation (SSI)(Malerba,2004), national systems of innovation (NSI) (Nelson, 1993; Lundvall 1992), and regional systems of innovation (RSI) (Cooke, 2001) – to simulate scientific, technological, innovative solutions within strategic sectors of both national and regional economies. Scientific discoveries facilitate the creation of new technologies; the outputs of which integrate and/or the application of these scientific and technological *development generates innovative solutions to be absorbed by individuals within civil society. Therefore, the developing of an adaptive STI capacity in any system of innovation is a function of advancements in science, technology, and an innovative-centric society. The opportunities for innovation through scientific discoveries and technological advances are endless. This author is postulating that, given the significance of innovation in promoting economic growth and social transformation to developing economies, then policies supporting innovation initiatives are necessary in all paradigm shifts involving socioeconomic transformation and national development of Caribbean economies.*

Keywords: STI Policy, Research and Development, Innovation, Economic Growth, Socioeconomic Transformation, SIDs

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Introduction

The economies of the Caribbean have long struggled with socio-economic development and long- term prosperity of its people for several decades. The peaks and troughs though minimal and often flat-lined in economic terms and social transformation have been talked about requiring much needed action by policy makers of the Caribbean. Economic development scholars (Schumpeter, 1942; Solow, 1956; Audretsch, 2004; Audretsch, 2007; Drucker, 1985; Conceicao, Gibson, & Heitor, 2000) have identified the link of innovation to the socio-economic development and prosperity of many nations especially in high-growth economies such as South-east Asia, Ireland, parts of Europe, and the USA (Kuen-Hung & Jiann-Chyuan, 2004).

Historically, one primary factor, the literature links economic development and social transformation of nations is innovation (Schumpeter, 1934). When used, the term economic development does not just translate to employment creation but refers to the continuous process of wealth creation for the people of a geographical area. The primary beneficiary of this phenomena is people of the locality with an overall outcome of social transformation and a build out of social capital. This interplay of dynamic economic activities with other social systems such as education, health etc generates a holistic and vibrant socio-economic environment facilitating business development opportunities with an administrative structure in the form of a firm. It is the firm which must be organized to facilitate absorption of modern scientific development and emerging technological advances based on their respective and collaborative absorptive capacities. These new and advanced scientific and technological advances may manifest as ***innovations – products, process, new paradigms or services***. Within the body of knowledge are several definitions of the term innovation. For the purposes of this conference paper, the author has adapted the following two definitions for innovation:

i. inventions and commercialization of new (or significant improvement of existing) products, processes and services (OECD, 2005).
ii. the application of "new technological or market knowledge to offer a new product or service to satisfy customers wants" (Afuah, 2003, p. 9).

A convergence of knowledge acquisition, the application of basic and applied sciences with advanced technologies generate several dimensions of innovations which can then be applied to various industries within a sector.

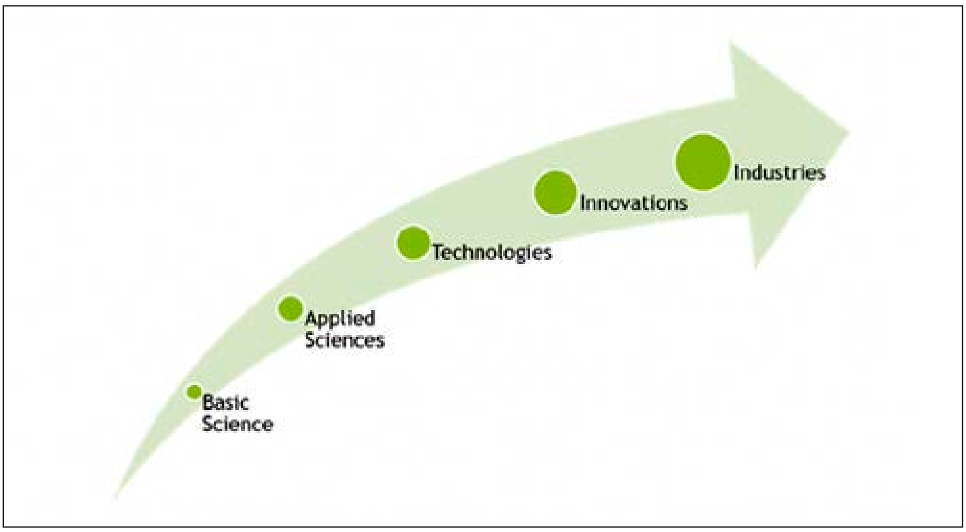

Figure 1: Progression of sciences to industries in strategic sectors

The emergence of the different types of innovations from the various forms the foundation to robust socio-economic growth.

Among the many critical success factors (CSFs) contributing to robust socio-economic development are:

1. Gross Expenditures in Research and Development (GERD) at both the micro and macro level.

ii. The nations' human capital development through its education and training programmes particularly in the sciences and technological advances.
iii. A well-defined, mature and sophisticated financial services network to facilitate efficient and effective financial transactions locally and internationally.
iv. A mature, robust infrastructure to support economic and social activities. These include though not limited to, reliable and cost-effective energy supply, an efficient well-defined transportation network, a reliable cost-effective information, communication and technological networks (ICTs] etc.
v. Development and empowerment of facilities to facilitate the effective and efficient transfers of scientific and technological developments both domestically and internationally.

Fundamentally, socio-economic development is based on the application of acquired basic and applied knowledge through various knowledge acquisition mechanisms. It is the transformation of these acquired knowledge which generates incremental, radical, disruptive and architectural innovations to the society.

Setting the Stage for a New Paradigm in the Caribbean

The territories of the Caribbean Region have been challenged with many socio-economic issues for decades. Like other regional blocs, though not of the same size, possess catch-up and/leapfrog opportunities in the revitalization of the respective economies through advances in science, technology and innovation (STI). The business development opportunities presented by emerging scientific developments and technologies in various sectors are endless.

Of significant importance to territories in the Region is breaking the barriers of entry into the Global Economy through the Global Value Chains (GVCs) mechanisms (Pietrobelli, 2022). This can advance the agenda of gaining and maintaining ***competitive advantage*** (Porter, 1990) status upon entry into one of the innovations domains.

For many decades various actors within and external to the region have advocated for a robust Science & Technology Policy. Such a policy will be the foundation to enhancing the absorptive capacity in innovation of the Caribbean Region, which can facilitate various Sectoral Systems of Innovation (SSIs) (Malerba, 2004) for various strategic sectors such as Tourism, Agriculture, Health, Education and Manufacturing. These primary sectors should be seen to have with many cross-linkages with other sectors and supporting systems to form a National System of Innovation (NSI) (Nelson, 1993; Lundvall, 1992; Freeman, 1981) at the

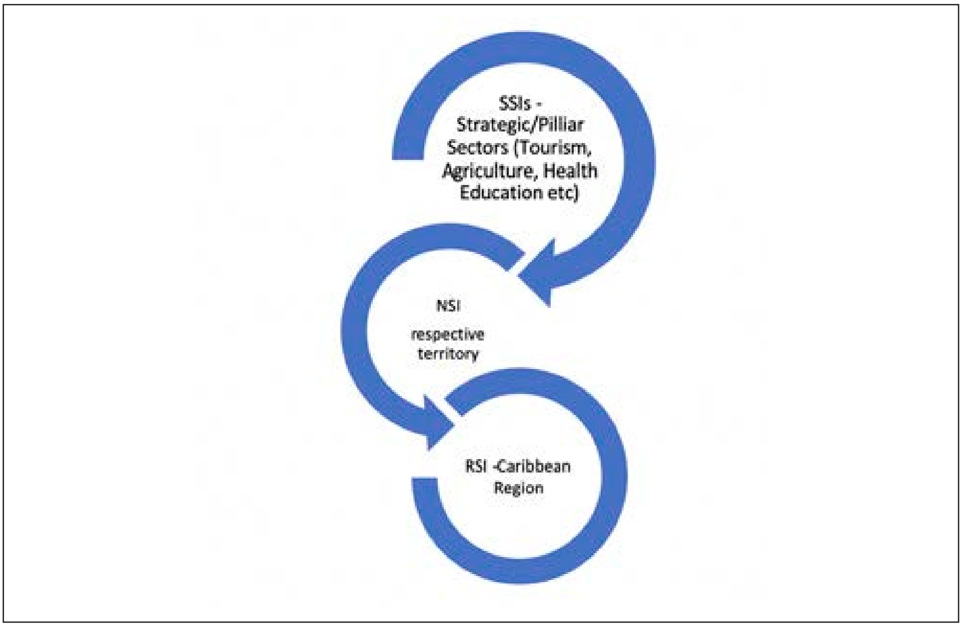

Figure 2: Schematic view of the Systems of Innovation

level of the territory. The synergies between the various SSIs and NSIs can amalgamate to form a Regional Systems of Innovation (RSI) (Cooke, 2001) for the Caribbean region.

The big challenge of the Caribbean Region is keeping abreast of the seasonal technological advances. The Region has faced what the literature referred to as the 'Red Queen Effect' (Paus, 2017). This "effect" is the need for middle income counties like those in the Caribbean to accumulate adaptive innovation capabilities (Spinolata, 2022) a rapid pace just to maintain a seat at the STI table.

Dr. The Rt. Hon. Keith Mitchell[1] in his keynote address[2] at the recently held FORECAST Conference[3] (August 10–12, 2022) led the charge for researchers, scientists, technologists, policymakers, private and public sectors inclusive of citizens of the Region towards the realignment of our thinking and action toward the advancement of STI in the Caribbean region. This he states will increase the prowess in the global economy.

1. Former Prime Minister of Grenada. Former Lead Head of Government in the CARICOM Quasi Cabinet with responsibility for Science and Technology, inclusive of ICT.
2. Science and Technology and its Application to Regional Development.
3. FORECAST CONFERENCE – A D.R.I.V.E.R (Development, Resources, Innovation, Vision, Entrepreneurship, Renewal) for Transformation.

Similarly, Emeritus Professor Ishenkumba Kahwa[4] in his plenary address[5], succinctly identified the dire state of affairs in terms of the Region's scientific research, any form of technological developments even at the minimum level for scientific high-tech products, processes, services and patents registration. For the calendar year ending 2020, the Region registered only 12 patents (CEPAL, 2020). Comparatively, that figure is a very low considering the correlation between R&D, intellectual property (IP) registrations and innovation intensity[6] of territories in the Region when compared with other regional blocs. Thus if territories within the Region are serious about innovation then, it is imperative that the required policies and related governance structures must be formulated, executed and monitored to advance STI development within the Region.

Our Caribbean region is often referred to as CARICOM, referring to the English-speaking territories. However, the Region should be referred to as the CARIFORUM to include the other territories who have other international languages as part of their culture. Here, the author refers to the Spanish-speaking neighbours of Cuba and Dominica Republic etc, French speaking as in Haiti, Martinique etc. The bloc of CARIFORUM territories can certainly leverage their respective uniqueness in natural resources, creative industries and other centres of excellence to advance SSIs, NSIs and the ultimate RSI for CARIFORUM. Such a proposition can generate several opportunities for the advancement of **competitive *advantage*** (Porter, 1985; Porter, 1990) within the global value chain (GVC). However, this will require structural changes to the education systems to include the development of multi-lingual skill sets and other areas necessary to advance the STI culture in the Region. Cuba as a Spanish speaking neighbour and the largest Caribbean territory, has been listed as generating the largest number of tertiary graduates in both basic and applied sciences. This territory invests approximately 16% of its GDP in education. This fact should be viewed as a competitive advantage to the Region especially in the human capital development for capacity building and the further development of knowledge transfers.

Associated risks factors, digital divides, gaps in technology adaptation, and the various stages of the digital transformation within territories are challenges to overcome. Is the development and implementation of a robust STI policy sufficient to advance science, technology and innovation towards sustainable

4. Professor of Supramolecular Chemistry, University of the West Indies.
5. Are CARICOM Countries innovating into the Fourth Industrial Revolution?
6. Innovation intensity refers to the commitment of a firm to innovation activities and is measured by the ratio of expenditure on innovation relative to the generation of income from various types of innovations.

socioeconomic progress and prosperity for Caribbean citizens the panacea for the Region? Is there a need to advance the agenda to create an ***entrepreneurial society*** (Audretsch, 2009b) as a foundation to further advance the STI agenda? What are the roles of the various ***actors*** in the formulation, implementation and monitoring of a robust STI policy for respective territory and the Region? How do stakeholders/actors (CARICOM, CDB, OECS, CARIFORM, etc) of the Region factor in the STI policy conversation? A necessary imperative.

The Case for STI Policies in the Region

Below is a non-exhaustive list (not in order of precedence) of territorial and regional challenges for STI Application.

- Climate Change & Resilience; Extreme Heat, Natural Disasters
- Education – Across all cohorts of learners; low literacy rates
- Crime & Violence – Human Trafficking (Women & Children)
- Poverty (Targeting Disadvantaged groups)
- Affordable, Cost-efficient Housing – Supply & demand
- Energy; Costs, Reliability
- Pollution
- Population
- Transportation Management; Impact on productivity
- Health Care Management; Individual & Macro-Level

The author refers to the primary actors in the establishment of Caribbean innovation-centric society; foundation for a RSI with an innate innovation-centric culture at each national level (NSI) to fuel the sustainability of the ***innovation fire*** that has started its burn. Some named actors (though not limited) to this innovation conversation include:

- National Institutions such as Development Banks, National Planning Agencies, Tertiary Institutions, Vocational Training Institutions.
- Public Sector Agencies; Ministries, Department & Agencies (MDAs) in respective Territory
- Private Sector – MSMEs – Alliances/Collaborations on Innovative Projects, Large Conglomerates
- Regional/International – NGOs – CARICOM, CDB, OECS, OECD, EU, IADB, OAS, World Bank, Multinationals
- STI –Campion(s) – National & Regional Level

Selected areas where a national and regional Science, Technology, and Innovation (STI) policy can have tremendous societal impact are:

- Climate Change & Resilience Management focusing on the management of the Water sector
- Environment Protection Management & Monitoring
- Food Safety & Security
- Expansion of Micro, Small- Medium Enterprises (MSMEs) especially in the creative and gastronomy industries
- Advance the development and management of Smart cities
- Building/Retrofitting for more Green and Eco-friendly Buildings for both Residential & Commercial occupants
- Creating greater opportunities for the Caribbean inclusion in global competitiveness by tapping into the Global Value Chains (GVC) – Niche Marketing; Market (Traditional & Emerging markets Penetration)
- Sophisticated and robust Financial Systems to fund innovative capital projects through grant funding and foreign direct investments (FDIs)
- Domestic & International Trading (Logistics Planning)
- Lifestyle/Behavioral Changes – Incentives on introducing electric vehicles – effect on the Region's carbon footprint, and
- Advance infrastructure Development towards productivity effectiveness & efficiency.

These are just a few industries for immediate application of science and technology from which a robust entrepreneurial society may develop (Audretsch and Thurik, 2001a; Audretsch, 2009b; Schumpeter, 1942).

Final Thoughts – Low Hanging Fruits for Immediate Action

- Encourage the formation of innovation-centric societies at local and regional levels. Such initiatives can begin with the formulation of SSIs in strategic sectors in each territory with cross-linkages to similar sectors in other territories. These SSIs can feed into the development of NSIs for each territory and again across territories; to form a RSI for CARIFORUM.
- Increase investments in Human Capital Development locally and regionally. This requires the necessary investments in knowledge creation and transfers modalities to support the STI ecosystems.
- Action by policy makers (actors/stakeholders) to formulate, execute and moni-

tor legislative and regulatory artefacts in the empowerment of an environment for advancing innovative Public-Private-Partnerships (PPPs) and facilitating the ability of attracting more foreign direct investments (FDIs)

- Formulate and execute policies to simulate and encourage the creation of a cohort of leaders who will lead innovative initiatives. This group should be perpetual and agile in nature for sustainability.
- Simulate and invest in R&D initiatives fostering the dimensions and types of innovations sectorally, nationally and regionally. A call is being made for respective governments of the Caribbean region to include R&D funding as part of the national budget; not subsumed under other line items but its own budget line to elevate its importance to national and regional development.
- Advance the formulation and monitorization of industry standards, code of ethics, quality management, of our outputs.
- An urgent appointment of STI campions at both the national and regional levels to advocate the STI agenda and policies development.

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Journal of Arts, Science and Technology

Bioprospecting and Biopiracy in the Caribbean

Challenges and Opportunities for the Region

SYLVIA MITCHELL
University of the West Indies, Mona

KEVEL LINDSAY AND **ANTHONY RICHARDS**
Wild Caribbean, Antigua and Barbuda

Abstract

The Caribbean is the seventh largest biodiversity hotspot (36 are recognized worldwide). This biodiversity is under myriad of threats including biopiracy, inertia, inequitable land use, and climate change. The loss of potential income due to biopiracy has made the Caribbean poorer as it has only benefitted countries outside of the region while leaving the Caribbean footing the bill for maintenance of its bioresource. The Convention on Biological Diversity (CBD) entered into force in 1993 and the Nagoya Protocol (NP) in 2014. The CBD, ratified by all UN members except the US, affirms that conservation of biodiversity is a common concern, reaffirms States have sovereign rights over their bioresources, while recognizing the close and traditional dependence of indigenous and local communities to this bioresource and the desirability of sharing benefits equitably with them for conservation and developmental purposes. The Nagoya Protocol provides a legal framework for access and benefit sharing when biodiversity, with or without associated traditional knowledge, from one party is sold as commercial products by another. As part of a recent UNEP project, which identified issues Caribbean nations face in ratifying and utilizing the NP, reported here are bioprospecting cases that have, or have not, benefitted the region. Included are biopiracy cases which had not but are now providing benefits to the Caribbean. Besides the obvious need for the Caribbean to invest more in valuing our own biodiversity and ratifying the NP, opportunities for the region include *keeping more of the bioprospecting value-chain in the Caribbean, converting value-chains into value-rings, and developing regional registries and databases.*

Keywords: Traditional Knowledge, Indigenous and Local Communities, Taino, Kalinago, Maroon, Bioresource, Nagoya Protocol, Benefit Sharing, Ethnobioprospecting, Ethnobiology, Ecopharmacognosy, Ethnobiotechnology

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ABBREVIATIONS

ABS = access and benefit sharing
ACP = African, Caribbean and Pacific Group of States
CBD = Convention on Biological Diversity
CIBH = Caribbean Islands Biodiversity Hotspot
GEF = Global Environmental Facility
GIZ = Deutsche Gesellschaft für Internationale Zusammenarbeit
GR = genetic resources
ILCs = Indigenous and Local Communities
IPR = Intellectual Property Rights
IUCN = International Union for the Conservation of Nature
MAT = mutually agreed terms
MTA = Material Transfer Agreement
NP = Nagoya Protocol
PIC = prior informed content
SIDS = Small Island Developing States
TK = traditional knowledge
UNEP = United Nations Environment Programme

Introduction

The Caribbean is of critical importance for global biodiversity survival due to its high percentage of global plant and animal endemics and the high level of threats. Of particular concern are concentrated areas of endemism (e.g. the Blue & John Crow Mountains which have 50% endemicity in the flowering plants at elevations above 900–1000 m asl with between 30–40% of these species found only within this region, https://whc.unesco.org/en/list/1356/). There is a cost in taking care of this biodiversity, to ensure it will still be here for future generations.

Caribbean Island Biodiversity Hotspot

The Caribbean Region encompasses 30 diverse nations and territories in over 7,000 islands, islets, reefs and cays with 230,000 km^2 total land area in ~4 million km^2 of ocean (CEPF 2019). The Caribbean contains the seventh largest of 36 recognized 'biodiversity hotspots' in the world (Myers 2000). This is referred to as the Caribbean Islands Biodiversity Hotspot (CIBH). To qualify as a biodiversity hotspot according to Myers (2000), a region must contain >1,500 species of endemic vascular plants (>0.5% of world's total) and have lost >70% of its primary vegetation (Myers 2000,). The CIBH has ~12,000 known plant species, of which 7,000 are endemic to the hotspot, 2.3% of global plants, and 779 endemic vertebrates while endemics are still being found (Figure 1; Anadón-Irizarry et al., 2010; Mitchell et al., 2019; https://ec.europa.eu/environment/nature/biodiversity/best/regions/caribbean_en.htm).

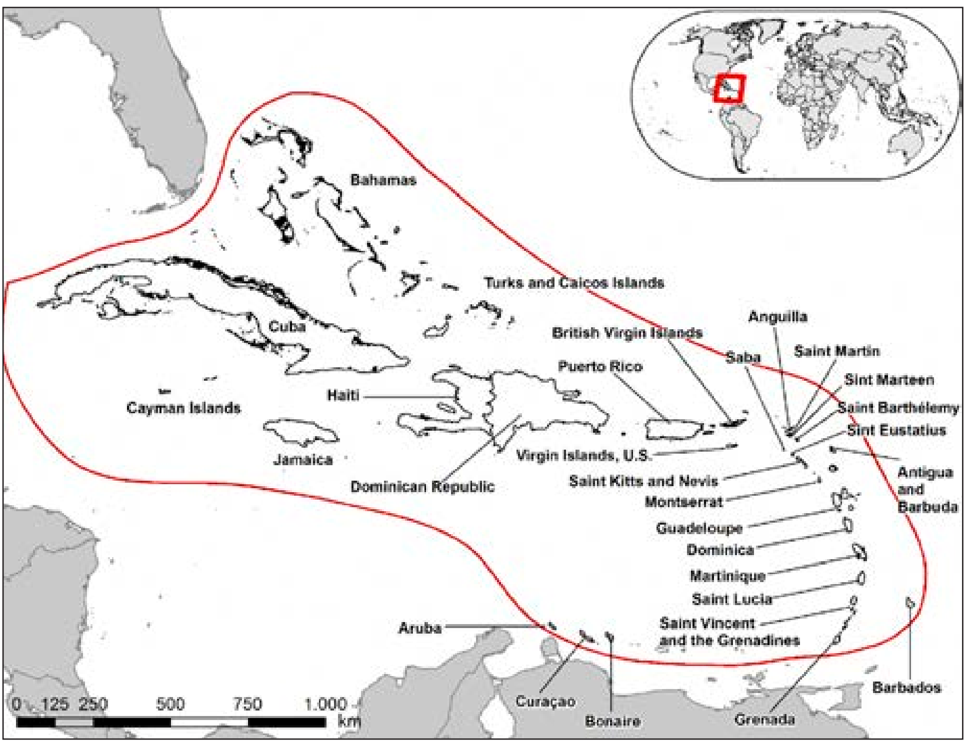

Figure 1: Caribbean Island Biodiversity Hotspot (CIBH)

Source: https://www.cepf.net/sites/default/files/cepf-caribbean-islands-ecosystem-profile-summary-2020-english.pdf

Threats to Caribbean Biodiversity

The highly valued genetic resources in the CIBH are under myriad threats due to the historically extractive nature of the plantationocene and its aftermath, which includes forest clearance, biopiracy, ignorance; inertia, unsustainable use, undervaluation of biodiversity; land degradation, pollution from nutrients, inequitable land planning, urbanization and climate change (Mitchell et al., 2007, 2008, 2019). These threats challenge the very existence of the CIBH endemics (Oleas et al., 2013). Endemics (plants and animals) have been found only in the CIBH; restricted to a few or one island; or even, only to one part of an island (Anadón-Irizarry et al., 2010). Endemic organisms are also still being discovered. The Caribbean is thus of critical importance for global biodiversity survival. While a lot of research has taken place (Oleas et al. 2013, Mitchell et al., 2019, CEPF 2019, 2022), a lot more research and development are needed. For example, Jamaica's only flora reference book lacks illustrations (Adams, 1972) and some forest plants are still unknown to science (Mitchell, 2011). Those that live in the Caribbean need to value its biodiversity and sustainably use it for health and wealth (Mitchell et al., 2018a). Corporations who still practice extractive research, leading to unequitable benefit sharing with the Caribbean, continue to threaten the sustainability of Caribbean biodiversity. In this paper, we will draw attention to the threat of biopiracy.

The Caribbean is the only major biodiversity hotspot comprised of many nations with varied affiliations (Table 1, Mitchell, 2019). This makes regional conservation and sustainable use management difficult but essential. How should the Caribbean sustainably manage its biodiversity given the challenges and opportunities of bioprospecting? When bioprospecting returns no returns to the country of origin, it is called biopiracy. To determine the threat of biopiracy to Caribbean biodiversity, it was necessary to evaluate for the Caribbean the present status of biodiversity knowledge, biodiversity threats due to biopiracy, and international biodiversity agreements including legal instruments such as the Nagoya Protocol which aim to share the benefits of commercialization of bioresources back to source countries for conservation purposes, and then consider what should be the Caribbean's response.

Conservation Conventions

Convention on Biological Diversity (CBD)

The United Nations Environment Programme (UNEP) tasked experts to prepare a legal internationally recognized instrument for conservation and sustainable use of biological diversity. They were to consider "the need to share costs and benefits between developed and developing countries" as well as "ways and means to support innovations by local people". The result was the Convention on Biological Diversity (CBD) which entered into force in 1993. The CBD is an international legal instrument for "*conservation of biological diversity, sustainable use of its components and fair and equitable sharing of benefits arising out of the utilization of genetic resources*". The CBD *affirms* that conservation of biodiversity is a common concern; r*eaffirms* States have sovereign rights over their biological resources and for using them in a sustainable manner, *recognizes* the close and traditional dependence of many indigenous and local communities on local biodiversity and *desirability* of sharing equitably benefits arising from use of this traditional knowledge relevant to the conservation of biological diversity and its sustainable use. For the full CBD agreement, see (https://www.cbd.int/doc/legal/cbd-en.pdf). The CBD has been signed by all UN members except the US. All Caribbean nations, except for the US-affiliated islands of Puerto Rico and US Virgin Islands, ratified the CBD between 1994 and 1997.

Nagoya Protocol (NP)

The NP was introduced in 2010 and entered into force in 2014 thus providing a legal framework for the Access and Benefit Sharing (ABS) aspect of the CBD. The NP framework requires prior informed consent (PIC) and mutually agreed terms (MAT) to include any Indigenous or local community (ILCs) that provided any associated traditional knowledge [TK]), between the country where the biodiversity was accessed (*country of origin*) and the party that produced derived commercial products from this genetic resource (*user*), resulting in agreed access and benefit sharing (ABS) terms. It obliges the user to share benefits with the country of origin. It was the first international instrument of particular relevance to ILCs since adoption of the UN Declaration on the Rights of Indigenous Peoples in September 2007 (Mauro and Hardison, 2000). Prior to the Nagoya Protocol there, were no legal mechanisms in place to ensure that countries, communities and individuals were properly compensated for the use of their genetic resources (GR) and associated TK. The NP offers an important opportunity for the Caribbean to ensure that use of its GR and associated TK benefits the region. This will help with management of the CIBH and development of the Caribbean countries with the accessed bioresources.

Caribbean Initiatives for Biodiversity Access and Benefit Sharing (ABS)

Two collaborative initiatives, led by Deutsche Gesellschaft für Internationale Zusammenarbeit (GIZ) between 2012 and 2015 (Period A) and UNEP between 2015 and 2019 (Period B) have helped Caribbean countries to understand, ratify and use the NP for biodiversity conservation and development. Many Caribbean countries, due to these initiatives and others, have ratified the NP, either on their own or by virtue of their relationship to a ratifying country (Table 1). For example, the Netherlands signed the protocol in 2016 so its overseas territories and special municipalities in the Caribbean, are also considered to have ratified the protocol.

Initiative 1 – ABS Capacity Development Initiative

The *ABS Capacity Development Initiative* (a multi-donor initiative hosted by the German Federal Ministry for Economic Cooperation and Development), has been implemented by Deutsche Gesellschaft für Internationale Zusammenarbeit (GIZ) GmbH since 2006. Following the adoption of the Nagoya Protocol in 2010, the European Union tasked GIZ to expand the ABS Initiative beyond Africa to include Caribbean and Pacific countries of the ACP (https://www.acpsec.org/). Between 2012 and 2015, GIZ hosted Caribbean meetings in Trinidad and Tobago, Dominica, Jamaica, St Lucia and Suriname (other island representatives were present) to discuss issues pertaining to the NP: its potential, challenges and opportunities while providing training in areas such as analysis of existing laws and developing drafting instructions for new laws necessary for its ratification (https://www.abs-biotrade.info). As a result of this effort and others, several Caribbean countries ratified the NP (Table 1, period A, https://www.cbd.int/abs/nagoya-protocol/signatories/).

Initiative 2 – UNEP 2015 Caribbean Project

A United Nations Environment Program (UNEP) project approved in 2015 entitled '*Advancing the Nagoya Protocol in Countries of the Caribbean Region*' (GEF ID

5774), was implemented between Febuary 2016 and March 2019 and continued this process. This project included ten countries (Antigua and Barbuda, Barbados, Dominica, Grenada, Guyana, Jamaica, St Kitts and Nevis, Saint Lucia, St Vincent and the Grenadines, and Trinidad and Tobago) with UNEP as Implementing Agency, the Global Environmental Facility (GEF) as funder, and the International Union for the Conservation of Nature (IUCN) as Executing Agency[1]. In the Caribbean, this GEF project financed at USD 1.826 million was supported by GEF-5 STAR funding of USD 1.900 million accessed by the Bahamas, to put in place the legal-, regulatory-, institutional- and administrative-systems needed to comply with the basic provisions of the Protocol. As a result of these initiatives and others, four more Caribbean nations ratified the NP (Table 1, Period B).

Table 1: Nagoya Protocol ratification status by Caribbean countries at December, 2022

	Country ratifying the NP	Date of ratification	Caribbean nation states covered by ratification
Period A	Guyana*	2014.10.12	
	Mexico*	2014.10.12	
	Dominican Republic*	2015.02.11	
	Cuba*	2015.12.16	
	Netherlands*	2016.11.17	Bonaire, Saba, Sint Eustatius (special municipalities); Curacao, Aruba, Sint Maarten (OCT)
	UK*	2016.05.22	Anguilla, Bermuda, British Virgin Islands, Turks and Caicos Islands, Cayman Islands, Montserrat
	France*	2016.11.29	French Guiana, Guadeloupe, Martinique, St Barthelemy, St Martin
Period B	Antigua and Barbuda*	2017.03.12	
	St Kitts & Nevis*	2018.12.04	
	Bahamas*	2022.03.30	
	St Lucia*	2022.06.12	
Nations that ratified the CBD between 1994 and 1997 are marked with an asterisk* The nation states in the Caribbean Region that have not yet ratified the Nagoya Protocol up to December 31, 2022 are Barbados*, Belize*, Dominica*, Grenada*, Haiti*, Jamaica*, St Vincent and the Grenadines*, Suriname*, Trinidad and Tobago* and the United States (Puerto Rico and US Virgin Islands).[2,3]			

OCT = EU Overseas Countries and Territories

1. See https://www.gefieo.org/data-ratings/projects/project-id-5774 and https://www.gefieo.org/sites/default/files/documents/projects/tes/5774-terminal-evaluation.pdf
2. https://www.cbd.int/abs/nagoya-protocol/signatories/
3. https://en.wikipedia.org/wiki/Caribbean_Netherlands

Initiative 3 – UNEP 2015 Caribbean Project Sub-component

As part of the UNEP project, a 3-member scientific team from the Caribbean (authors of this paper) carried out a component entitled '*Access & Benefit Sharing (ABS) – Bioprospecting in the Caribbean Region*'. This component explored Caribbean bioprospecting in relation to the Nagoya Protocol (NP). Other components of the UNEP project addressed capacity building for the development of legislation, information technology management, the engagement of policy makers, and ensured the participation of indigenous and local communities (ILCs).

Outputs of the *ABS – Bioprospecting in the Caribbean Region* component included a summary of the bioresource of each of the eight contributing nations (Mitchell et al., 2018a), its inter-connectivity with indigenous & local communities (ILCs), including Amerindians and Maroons (Mitchell et al., 2018b), mapped Caribbean ABS institutions (Mitchell et al., 2018c), recommended standardized methodology for Caribbean national registers of their biological resources (Mitchell et al., 2018d), valued Caribbean biodiversity knowledge (Mitchell et al., 2019), developed a roster of regional ABS experts, initiated a Caribbean medicinal plant database and produced ABS info-sheets for tour operations, policy makers and biosafety officials while collecting and discussing Caribbean bioprospecting cases (Mitchell and Richards, 2022).

This paper reports on cases of terrestrial and marine bioprospecting unearthed during the UNEP 2015 project which were initiated in the Caribbean, any known associated traditional knowledge, and any identifiable derived benefits for the Caribbean. Included are biopiracy cases that have only recently produced a benefit for the Caribbean. From analyzing these cases, we have identified challenges to be overcome and opportunities to be realized for the Caribbean Region. In forecasting for the future, besides the obvious need for the Caribbean to invest more in increasing knowledge about our own biodiversity and ratifying the NP, opportunities include developing more links in the bioprospecting value-chain in the Caribbean, converting the value-chains into value-rings, and developing regional registries and databases.

Methodology

An important aim of the *ABS–Bioprospecting in the Caribbean Region* component was to identify bioprospecting issues which could affect ratification and use of the NP in the region. The methodology included collection of desk and field data (from country visits, questionnaires, internet searches, government offices and data sources, academic institutions, and patent repositories), which were then discussed during seven project workshops (in Trinidad & Tobago, Jamaica, Barbados, St Kitts), During the workshops, validated findings of each project component were presented, the implications of ratifying the NP shared, and training carried out. The workshop participants included government environmental officers, academics, lawyers, patent officers, ILC representatives and representatives of CARICOM and IUCN. The outputs developed by each component were used by the larger project to assist Caribbean nations understand, prepare, ratify and use the Nagoya Protocol. Some of these findings relevant to bioprospecting and biopiracy in the Caribbean will be given in this paper.

Results

This section begins by defining bioprospecting and associated terms. We then describe historical Caribbean bioprospecting and the present status of terrestrial and marine Caribbean bioresource including identification of areas with special potential for bioprospecting. The section ends with a typology of bioprospecting cases, and a short review of three Caribbean IPR patent systems.

Defining Bioprospecting and Biopiracy

As the team delved into the literature and talked with governments, academia, regional conservation groups and indigenous leaders, it was clear that terminology needed to be mutually understood so the key terms are defined here. Bioprospecting is the exploration of biological material for its commercially valuable biochemical properties. When bioprospecting includes how indigenous and local communities (ILCs) use the local biodiversity (called ethnobiology, folk knowledge, or traditional knowledge = TK), the correct term to use is ethnobioprospecting. The bioprospecting value-chain begins with access to genetic resources and any associated TK, through research that usually includes patenting, and ends with its derived commercialization. Patents are usually sought to protect the claim for the user. Though bioprospecting began as a search for drugs to cure new diseases, bioactive compound research has verified many pharmaceutical, cosmetic, biotechnological, agrochemical, and food applications (Mateo et al., 2001 and Santana et al., 2021). Consider turmeric, exploring its biochemicals for use as an anti-inflammatory agent or for COVID (Lantz et al., 2005 and Babei et al., 2020) would be considered bioprospecting but using it to make curry for supper would not. This is lucrative business, more than half of developed drugs approved by the US FDA are derived or bioinspired from compounds obtained from living organisms (Santana et al., 2021).

Biopiracy is a purposefully contentious term, used to describe bioprospecting cases where biological material is accessed from a country without payment or returning any benefit. Of special concern is biopiracy of local folk knowledge (TK) without reward or benefit to the communities where the TK was accessed (Roht-Arriaza, 1996, Robinson, 2012, Mauro and Hardison, 2000). The loss of potential income due to biopiracy has made the Caribbean poorer as it benefits only countries outside of the region, leaving the Caribbean footing the bill for upkeep of its bioresource. Many authors have tried to define biopiracy, to determine its degree of occurrence, and why it is still happening (Crosby, 1986, 2003; Shiva, 1999, 2007; Mulligan et al., 2000; Merson, 2000; Mateo, 2000). However, no literature could be found from Caribbean scholars about bioprospecting or biopiracy as experienced by the region. The authors of this paper therefore started to explore the topic by asking a simple question: 'In cases where users accessed biodiversity in the Caribbean, have the region's communities derived any commercial or non-commercial benefit?' Based on the cases unearthed, another question was asked, 'What should be the Caribbean response given the history of biopiracy in the Caribbean and the opportunities possible through the Nagoya Protocol for conservation and developmental purposes?'

Caribbean Bioprospecting

Historical Caribbean bioprospecting

There is evidence that Amerindians (such as the Ciboney, Taino and Kalinago)) were in the Caribbean since at least 2,500 BC (referred to as Indigenous people due to how long they were present) and while sizeable populations occupied the islands, the natural vegetation was still described as luxurious centuries later[4]. When Europeans arrived, who did not know the Caribbean existed before 1492, they cleared the forests of many Caribbean islands to establish mono-crop plantations; this initiated a period referred to as the plantationocene (Wolford, 2021).

4. See https://www.pitonbungalows.com/en/the-first-residents-of-the-archipelago-the-amerindians/

The felled trees and other forest products (mahogany, bitter wood, cocoa, herbs etc.) and later sugar, bananas, coffee etc. were exported as raw material to Europe where they were made into value-added products (white sugar, chocolates, furniture, pharmaceuticals). This restriction of Caribbean colonial trade to export of raw materials and import of finished goods was largely influenced by the British Navigation Acts. In operation between 1651 and 1849, these Acts restricted British colony exports to raw materials and only to Britain, local manufacturing was discouraged, and colonies could only purchase manufactured goods from or through Britain (Fernandez-Villaverde, 2022).

Bioprospecting was an integral part of European exploration as new areas brought new diseases and plants found in these areas were being used medicinally by local folks (Crosby, 1986, 2003). The earliest known medicinal plant compendium was written in 2,500 BC and it listed 366 plant drugs (Merson, 2000)[5]. In the Caribbean, the botanist who described the most plants (including medicinal ones) was Sir Hans Sloane (1660–1753). He collected Caribbean plants and associated folk knowledge without acknowledging his sources nor providing any benefit to any Caribbean source country. His plant collections form the basis of the Royal Botanic Gardens Kew collection in the UK (Nesbitt, 2018). The Kew collection and many others contain Caribbean plants and associated traditional knowledge (TK), but they are outside the Caribbean region (in databases, herbariums, botanical gardens, pictures and paintings) and are still largely inaccessible to the Region. The challenges and opportunities resulting from bioprospecting and biopiracy endeavours in the Caribbean will be discussed, including how this inequitable situation has, and still is, hindering Caribbean scientists (Mitchell et al., 2019).

Present status of Terrestrial and Marine Caribbean Bioresource

Though extensive research has adequately described Caribbean terrestrial and marine bioresource and endemics specifically, and the need for conservation is well appreciated (Maunder 2008; Mitchell et al., 2018a,c, 2019; CEPF 2019, 2022), there is still insufficient knowledge especially as it pertains to medicinal plants of the region, their folk use and their conservation needs. Caribbean bioresources suitable for bioprospecting and associated TK were reviewed in this project – for terrestrial, fresh-water and marine; for flora, fauna and microbes (Mitchell et al., 2018a,b, 2019). As an example, an ethnobotanical study in Montserrat alone

5. Written by Emperor Shen Nung, the Father of Chinese medicine, circa 2500 BC.

reported 256 medicinal and 24 poisonous plants (Brussel, 1997). A Caribbean medicinal plant database was developed for this project. This database presently includes 3,566 medicinal plant species in the Caribbean identified as having at least one ethnomedicinal use, for 17 countries, from 34 source documents (Mitchell et al, 2019). This project database, along with the TRAMIL database (https://tramil.net/en) and others (https://www.dcbd.nl/, https://dcnanature.org/caribbean-biodiversity-on-line/) point to Caribbean terrestrial plant biodiversity as being a shared resource.

The exploration of the Caribbean marine bioresource has been researched, in patches, and there are a lot more areas to explore. Representative data has been stored in an international and open database (https://obis.org/). The Caribbean marine bioresource which is shared among the Caribbean islands (Mitchell et al., 2019) is of increasing interest as a source of bioactive compounds. Pharmaceutical active agents obtained from marine sites include anti-neoplastic agents, cardio-vascular active drugs, marine toxins, antibiotic substances, anti-inflammatory and antispasmodic agents[6].

Caribbean Sites with Special Potential for Bioprospecting

Most bioprospecting in the Caribbean has taken place in accessible areas, such as lowland forests, wetlands and near-shore marine, mangrove, reef and sea grass beds. Less well explored environments include volcanic hot springs, pitch lakes, marine environments at extreme depths, specifically around submarine trenches, cold seeps, seamounts and deep-sea hydrothermal vents. The biological processes which enable 'extremophiles' to survive extreme temperatures, pressures, salinity and so on have great potential for commercial applications. Enzymes derived from extremophiles, for example, have been used in detergents, cleaning, dyeing, medical diagnosis, cosmeceuticals, and for forensics.

Types of Bioprospecting Cases Originating in the Caribbean

Several bioprospecting cases were unearthed where the biological materials were accessed from within the Caribbean Region. More drugs were identified that have been developed from Caribbean marine species than from terrestrial species. Research also appears to be shifting away from invertebrates (corals, sponges, tunicates) to the associated algal symbionts and bacteria. The focus of research

6.w See https://www.slideshare.net/SudheerKandibanda/marine-drugs-56601492 has been cancer treatment. Molecular biology techniques are increasingly used to explore these microbial communities (genomics, proteomics). Six types of bioprospecting cases originating in the Caribbean were identified. The cases given here are by no means exhaustive but are meant to be indicative.

Type 1: Bioprospecting R&D outside Caribbean leading to commercial products based on Caribbean Bioresources, which has no recorded benefit to the region.

This type of bioprospecting was difficult to unearth. Caribbean biodiversity might have been accessed illegally from the region, or legally by transfer between botanical gardens, or via research projects using material transfer agreements (MTA). The best-known case is the Madagascar periwinkle which was accessed from Jamaica then put into trials in the USA and Canada, first for diabetes then for cancer. Two Jamaican medical professionals in the 1950s sent parcels of periwinkle material to Florida and Canada for these trials. Eli Lily, who eventually obtained patents for anti-leukemia effects of vincristine and vinblastine extracted from the periwinkle acknowledge no wrongdoing and only admit to receiving their first sample from Jamaica (Desai, 2011). Other cases are given in Table 2.

Table 2: Summary of unearthed Type 1 Caribbean bioprospecting cases

- *Tectitethya crypta*, is a marine sponge found in shallow water across the Caribbean. It was accessed first in 1945 by a young organic chemist, Werner Bergmann from the coastal waters of Florida. Scientists isolated two nucleosides from the sponge, spongothymidine and spongouridine, which were used as models for the development of anti-viral and anti-leukemia drugs. The first was approved by the FDA in 1969 and the latest, remdesivir, was found to be effective against SARS, MERS and COVID-19 (https://ocean.si.edu/ocean-life/invertebrates/sea-sponge-hiv-medicine).
- Ecteinascidin 743 was isolated in 1990 from the sea squirt *Ecteinascidia turbinate.* It can be found in shallow waters off the east coast of Florida, Bermuda and the Gulf of Mexico from the Caribbean Sea. Antitumor activity of *E.* turbinate extracts was reported since 1969 but exactly where it was first accessed and by whom is unclear. Ecteinascidinen is presently undergoing trials for use as an anti-cancer medication.
- Fer-de-lance venom, *Bothrops caribbaeus*, St Lucia (Gutierrez, 2008). The snakes *B. caribbaeus* and *B. lanceolatus* are endemic to the Lesser Antillean islands of St Lucia and Martinique. They are rare, highly venomous snakes. Venoms offer potential for new discoveries (https://edition.cnn.com/2015/07/15/health/deadly-venom-saves-lives/index.html). Although rare in the insular Caribbean, representatives of this genus of snakes can be found across tropical Americas, including CARICOM countries such as Trinidad & Tobago, Guyana, Suriname & Belize. The venom may have medical potential. Unauthorized access was obtained in the 1990s.

Table 2: Summary of unearthed Type 1 Caribbean bioprospecting cases *(cont'd)*

• Crassin acetate from Caribbean Gorgonian *Pseudoplexaura porosa.* Crassin acetate, a cyclic diterpenes observed to be comparatively inert to the mammalian system but extremely cytotoxic to human leukemic cells in vitro and mouse fibroblasts (https://www.slideshare.net/SudheerKandibanda/marine-drugs-56601492). Patent for use of crassin acetate as an analgesic was granted in 1972.
• In 2015, there was opposition to a patent awarded to French Institut de Recherche pour le Développement (IRD) for the antimalarial properties of simalikalactone E isolated from *Quassia amara*. The scientists interviewed members of Kali'na, Palikur and Creole communities in French Guiana (ILCs) and others then focused on that species. The IRD did not mention these ILCs as a TK source in its patent application filed 2010.06.17 and granted 2015.03.04. No certificate of compliance was filed. A 'First worldwide family litigation' was filed 2015.10.23. Presently the patent is not in force and anticipated expiration date is 2030.06.17. In 2016, IRD stated it will work out a protocol with FG to guarantee fair sharing of the scientific and economic benefits if the drug makes it to the market and ensure that people in French Guiana get it at an affordable price (https://patents.google.com/patent/EP2443126B1/en, Bourdy et al., 2017, https://www.science.org/content/article/french-institute-agrees-share-patent-benefits-after-biopiracy-accusations,).

Type 2: Bioprospecting R&D outside Caribbean leading to commercial products based on Caribbean bioresources, which has a recorded benefit to the region.

This type of bioprospecting was the most difficult to unearth. The only scenario under which this seems to have occurred is if a Caribbean national undertook research of a Caribbean plant outside of the Caribbean, then returned to patent and develop commercial products. Thus, that researcher would be the one benefiting the region as long as they stay in the region. The best example unearthed of this is Dr. Lawrence Williams who dedicated his life work to researching one plant, guinea hen weed (*Petiveria alliacea*). He received financial support from Caribbean business interests and in the process developed an anti-cancer drug. Two products on the market are *Guinea Hen Weed Tonic and Restorative* and the *Guinea Hen Weed Magic Relief pain ointment.*

Type 3: Caribbean Bioprospecting R&D begun in the Caribbean leading to commercial products, which has no recorded commercial benefit to the region.

This has been the most typical type of Caribbean bioprospecting. A review of medicinal plant research in the Caribbean indicated that 334 plant species growing in Jamaica had at least one folk medicinal use, 193 of these have been tested for their bioactivity, crude extracts from 80 plants had reasonable bioactivity and phytochemicals were being identified from 44 plants (Mitchell and Ahmad, 2006). This and other literature indicate ethnobioprospecting is being done by regional scientists (Cohall, 2010; Picking et al., 2011, 2015; Mitchell, 2019) but further research leading to patentable claims has been, and is still being, done outside the region leading to patents being assigned to external researchers. The larger islands, Cuba, Jamaica and Puerto Rico can carry out clinical trials but resources do not permit the whole value-chain to market to take place in the Caribbean. The one exception to this is Cuba, which has been able to complete the entire value-chain as seen under Type 4.

Type 4: R&D begun within region leads to products based on Caribbean Bioprospecting, which has recorded benefit to the region.

As with Type 2, only a few cases were found for this type of bioprospecting. In one case, all the research was carried out in the Caribbean, by Caribbean academics. The final product was registered but no patent applied for. This means the details of the innovation remain a tightly kept trade secret to this day. This is the story of Canasol (registered as a Drug in Jamaica in 1983) and Asmasol (registered in 1990) extracted from *Cannabis sativa* by two Jamaican scientists Drs West and Lockart. Canasol is used to treat glaucoma and Asmasol to treat coughs, colds and bronchial asthma. Neither are protected by patents. Both products are sold only in Jamaica.

In another case, research in Cuba led to a medical product from Blue or Red scorpion, *Rhopalurus junceus* which can be found in Cuba and Dominican Republic. Blue scorpion venom research done in Cuba resulted in Vidatox 30 CH, which has been described as Cuba's newest homeopathic medication (https://cubamedicos.com/products/vidatox).

Type 5: Bioprospecting, where eventually a benefit was realized for the Caribbean due to compliance with the Nagoya Protocol.

A Type 1 case was unearthed where a benefit was eventually secured by a Caribbean country (The Bahamas), from which the original material was accessed (Table 3). This story started off with an inequitable bioprospecting relationship: the University of California (UC) collected soft corals (*Pseudopterogorgia elisabethae*) in

Bahamian waters, discovered bioactive pseudopterosines and obtained a patent for a derived anti-inflammatory product all without a benefit-sharing arrangement with the country of origin (The Bahamas). UC declined in the 1990s to sign an access and benefit-sharing (ABS) agreement with the Bahamian government. However, The Bahamas later enforced benefit sharing in 2001 (Table 3), authoring only a single company to access (harvest) the soft coral. The limited benefits under this agreement has supported surveys, conservation education and resource management, which was needed for sustainability of the resource(http://wings.buffalo.edu/academic/department/fnsm/bio-sci/faculty/lasker.html). However, the relationship remains inequitable, as the Bahamas obtained no benefit from the value chain.

Table 3: Bahamas soft coral bioprospecting story

In a nutshell • **1982**: Sampling of soft corals in The Bahamas by the University of California (UC) • **1986**: Anti-inflammatory properties of pseudopterosines published by UC • **1988**: U.S. patent on pseudopterosines and synthetic derivatives granted • **1990s**: OsteoArthritis Sciences Inc. tested methopterosin in phase I and II clinical trials • **1995**: Estée Lauder started to use coral extracts in cosmetics, licence fees for UC 750.000 USD per annum. More companies followed. • **1990s**: University of California declined entering a benefit-sharing agreement with The Bahamas • **2001**: Benefit-sharing agreement concluded between Government of the Commonwealth of The Bahamas, the local company Marsh Harbour Exporters and Importers Ltd. and the U.S. company Lipo Chemicals • **2014**: Close to 1 mill USD paid into a fund for surveys, conservation education, and resource management • **2014**: 145 cosmetic products use coral extract from The Bahamas, 50% of these products belong to Estée Lauder • **2015**: The Bahamas started UNEP GEF ABS project • **2022:** Bahamas acceded to the Nagoya Protocol

Type 6: Bioprospecting originating in the Caribbean with derived products still in the pipeline.

There are bioprospecting cases that begin with accessing a Caribbean bioresource which are still in development. It is unclear whether any of them have negotiated an ABS agreement between the country of origin and the user as required by the Nagoya Protocol. An example is plitidepsin (I-II) extracted from the anscidian *Trididemnum solidum*. This ascidian is present throughout the West Indies from Florida and Bahamas to Venezuela. The patent states that the bioresource was accessed from Belize, Colombia, Honduras, Mexico, Nicaragua, and Panama. There are also several New Marine Natural Products from Invertebrates (NMNPI) accessed in the Caribbean. In 2015, the Total NMNPI identified in Bahama waters exceeded the total identified in the rest of CARICOM (MarinLit database http://pubs.rsc.org/marinlit/).

Intellectual Property Protection Levels in the Caribbean

Commercializing biodiversity-derived products usually involves obtaining a patent for the use claim. To be NP compliant, countries have to ensure their Patent Offices are informed about the origin of any genetic resource accessed and any ILC that provided TK, before a certificate of compliance is given from a competent country authority. Therefore, to determine if the Caribbean is ready for ratification of the NP, the status of national patent laws (Table 4) and the number of patents (Table 5) was reviewed. For the sake of this paper, only three countries are reported but the databases can be searched for other countries.

Patent level protection in the Caribbean is low but increasing (Table 4). Patent laws have been updated (e.g., the old Patent Act 1857 of Jamaica was replaced in 2020) but more legislation is needed especially to cover micro-organisms. In terms of the number of patents, which suggests the level of ongoing research, those filed by residents were much lower than those filed by non-residents

Table 4: Status of relevant Patent Laws

Country	Signatory to International Law
Barbados	Barbados *Patent Law* of 2001 was amended 2006. Barbados has been a signatory of the *Patent Cooperation Treaty (PCT)* since 1985 but not yet to the International Union for the Protection of New Varieties of Plants (UPOV).
Trinidad & Tobago	The Trinidad and Tobago (T&T) *Patent Law* of 1996 was amended in 2000. T&T is signatory to the *PCT* since 1994, the *Budapest Treaty on the International Recognition of the Deposit of Microorganisms for the Purposes of Patent Procedure* since 1994, and the *Strasbourg Agreement Concerning the International Patent Classification* since 1996.
Jamaica	In Jamaica, the new *Patent and Design Act*, 2020 replaced the Patent Act, 1857 and the Designs Act, 1937. This allowed Jamaica to finally sign the PCT in 2022. The PCT makes it possible to seek patent protection for an invention simultaneously in numerous countries by filing an “international” patent application. The PCT now covers 153 contracting states. Jamaica is not yet signatory to UPOV nor the Budapest Treaty.

https://wipolex.wipo.int/en/members/profile/

Table 5: Number of Patents filed between 1960–2020 (World Bank database)

	1960–1979	1980–1999	2000–2009	2010–2020	TOTAL
Local residents[a]					
Barbados	0	3	6	33	42
Trinidad & Tobago	0	17	105	27	149
Jamaica	0	75	119	201	399
Non-residents[b]					
Barbados	0	185	771	389	1,345
Trinidad & Tobago	0	946	2,130	1,398	4,474
Jamaica	0	778	897	866	2,541

[a] https://data.worldbank.org/indicator/IP.PAT.RES
[b] https://data.worldbank.org/indicator/IP.PAT.NRES

(Table 5). A small but increasing number of drug-related patents have been awarded to Caribbean researchers (as suggested by the increase in local patents in more recent years in Barbados and Jamaica). In Jamaica, patents were awarded for products derived from terrestrial herbs used in traditional healthcare, achieved through collaboration with universities in North America.

Discussion

The authors of this paper asked a simple question: 'In cases in which users accessed biodiversity in the Caribbean, have the region's communities derived any commercial or non-commercial benefit?' We attempted to answer this question by exploring the types of bioprospecting cases that have occurred in the Caribbean and presented three types of cases (Types 1, 3, 6) where there is no recorded benefit, and three (Types 2, 4, 5) that had some recorded benefit to the region. However, none of the cases unearthed had an ABS agreement as envisaged by the Nagoya Protocol.

The second question we will attempt to answer in this section: 'What should be the Caribbean response given the history of biopiracy in the Caribbean and the opportunities possible through the Nagoya Protocol for Caribbean conservation and development?' We will attempt to answer this question by enumerating the challenges facing the Caribbean in utilizing the Nagoya Protocol and suggest some opportunities that should lessen or eliminate biopiracy by enabling sharing of benefits derived from bioprospecting. We will explore how the Nagoya

Protocol and other initiatives can help the region. Given the possibility for access and benefit sharing under the Nagoya Protocol, this is where our discussion will primarily be based.

Challenges of Providing Access to Caribbean Genetic Resources and Traditional Knowledge and Realizing Benefits of this Bioprospecting via Utilization of the Nagoya Protocol

There were many challenges identified that have been slowing the process of ratification of the Nagoya Protocol in the Caribbean (Table 1). Challenges were also identified which will hinder or slow the use of the Nagoya Protocol. During project discussions, a general lack of understanding of, and belief in, the potential of the Nagoya Protocol was expressed by several Caribbean nations. A challenge identified, which happened on more than one occasion, was that officials vested with such understanding through the GIZ and UNEP projects from 2012 to 2019, had since left critical government posts, resulting in the process towards ratification being stalled or having to be restarted. A clear message needs to be conveyed and understood – that biodiversity-rich countries become relatively poorer when they provide access to their GR and TK and do not secure a reward but still spend on conservation, while at the same time other countries benefit from selling products derived from said GR/TK without the conservation bill. This is an ongoing process due to the entrenched plantationocene systems. Genetic resources (GR), with or without TK, from the Caribbean are still being developed into manufactured products by extra-regional companies without sharing benefits with the Caribbean (biopiracy).

Even with understanding the potential of the Nagoya Protocol, there are several requirements a country needs to ensure they are compatible with the Nagoya Protocol before ratification is possible. These include reviewing existing laws, training people to provide drafting instructions of any required new laws, training in writing agreements with mutually agreed terms (MAT) and prior informed consent (PIC), possibly updating patent laws, carrying out stakeholder meetings while developing a system for issuing certificates of compliance with the Nagoya Protocol. This process takes time and human resources for which there are many other competing matters. Governments need to be convinced of the benefits of ratifying the NP before they will allocate time and energy in the process (even if external funds and expertise is available).

Challenges encountered with using the Nagoya Protocol in the Caribbean

- Accessing a bioresource in the Caribbean is problematic for benefit sharing because the same genetic resource (say a plant) may be on different islands governed by a different country. Access is not about where a plant or animal is endemic (originally found) but where it is was first accessed. However, a researcher may have an ABS agreement with the country where the genetic resources (GR) was first accessed, but when needing it again further along the value chain, may access it from another Caribbean country with whom they do not have an ABS agreement.
- Challenges exist with identifying Indigenous and local communities (ILCs) in the Caribbean. It is generally agreed that ILCs in the Caribbean include the Taino, Kalingo and Maroons but this term can also include any community that holds folk knowledge in common.
- Indigenous and local community members expressed challenges with prior informed consent (PIC) and mutually agreed terms (PIC) required by the NP. Ethics bodies require of researchers only individual PIC. Since this project, knowledgeable researchers have begun to obtain PIC from ILCs before interviewing individuals (who also are asked to provide their own PIC) while knowledgeable ILCs have developed terms of engagement with researchers. These are promising developments.
- A system needs to be in place to assist ILCs produce legally binding agreements to prevent lopsided agreements. Such an agreement would include suitable mutually agreed terms (MAT) before access is allowed, and again before utilization takes place. Such a system should be developed by ILCs, ethics bodies, academia, government officials, and the competent authority, within a country and between Caribbean countries. Allowing bioprospecting to occur in a country without these legal documents, hinders sharing of benefits.
- Even with PIC and MAT in place, to ensure ABS, the patent system needs to ensure that a certificate of compliance to the NP is obtained before the patent can be granted. A certificate of compliance needs to be issued by a competent authority. It should record date and place GR was accessed, description of GR utilised, source from which GR was directly obtained, name of any accessed ILC, any associated TK, names of any local informants, names of any local scientists involved in accessing the GR, access permits where applicable, presence or absence of rights and obligations relating to ABS and obligations regarding subsequent application and commercialisation, ethical approval of individuals and any ILCs involved, due diligence declaration to a relevant

authority upon receipt of research funding and at the final stage of development of a product. To be NP compliant means users must do everything within reason to at least identify the country of origin of the GR they intend to use. This will allow tracing back to source when benefits are to be shared.

- During ethnobotanical studies, local informants are usually not included as co-authors, often only the external researcher, who came into the region for only a short period of time, is the only author on the paper, leaving out even local researchers who assisted with of the study. It should be required that local researcher(s) be involved with local bioprospecting projects and that they be listed as co-authors in any derived publications. This is especially so when external researchers are only in the region for a short period of time and depend on local researchers for help getting around, obtaining ethics approval etc.
- Other weaknesses in the system that need to be addressed include but are not limited to the following:
    - Consultants, Academia and Government regulators in the Caribbean often have no access to or membership in existing global natural product databases. This is especially limited in the smaller islands.
    - General and specialist information capacity is limited among regulatory agencies, customs officers, and other relevant bodies.
    - Taxonomy training is insufficient and of limited interest to students, herbariums are under-funded and under-staffed, flora and fauna guides insufficient.
    - Limited local capacity for exploration especially of marine and terrestrial invertebrates. Limited capacity in submersible.
    - How can we arrange the IPR system to include community-TK?
    - Who will foot the bill if litigation is needed between the country of origin and the user?

Opportunities Identified for Realizing Benefits Accruing from Bioprospecting in the Caribbean

Five identified opportunities for gaining equitable benefits from Caribbean biodiversity will be shared in this paper. The first and most important opportunity is to ratify the NP so it can be used for those cases for which it applies. The second is to use ecobioprospecting and ethnobiotechnology to obtain more benefits throughout the value-chain. The third is to turn value chains into value rings.

The fourth is to develop alternative IPR regimes such as copyleft and geographical indicators to gain sustainable benefits from regional biodiversity. The fifth is to develop a regionally accessible and standardized bioprospecting database. To explain these opportunities, it is necessary to introduce a new version of the value-chain. These opportunities forecast a roadmap towards a more equitable, sustainable, economically viable biodiversity-rich future for the Caribbean.

Proposed Value-Web for Biodiversity Hotspots

The typical value-chain for bioprospecting was developed by users and not by the country of origin, where GR is accessed. It pre-supposes that the GR collected at the beginning of the process from the country of origin, will be available when R&D is complete, and material is needed for mass-prıoduction of drugs. It also assumes that manufacturing will occur in the user country.

Just as food-chains are better viewed within a food web, value-chains are better viewed in a value-web as proposed in Figure 2. This figure models all the

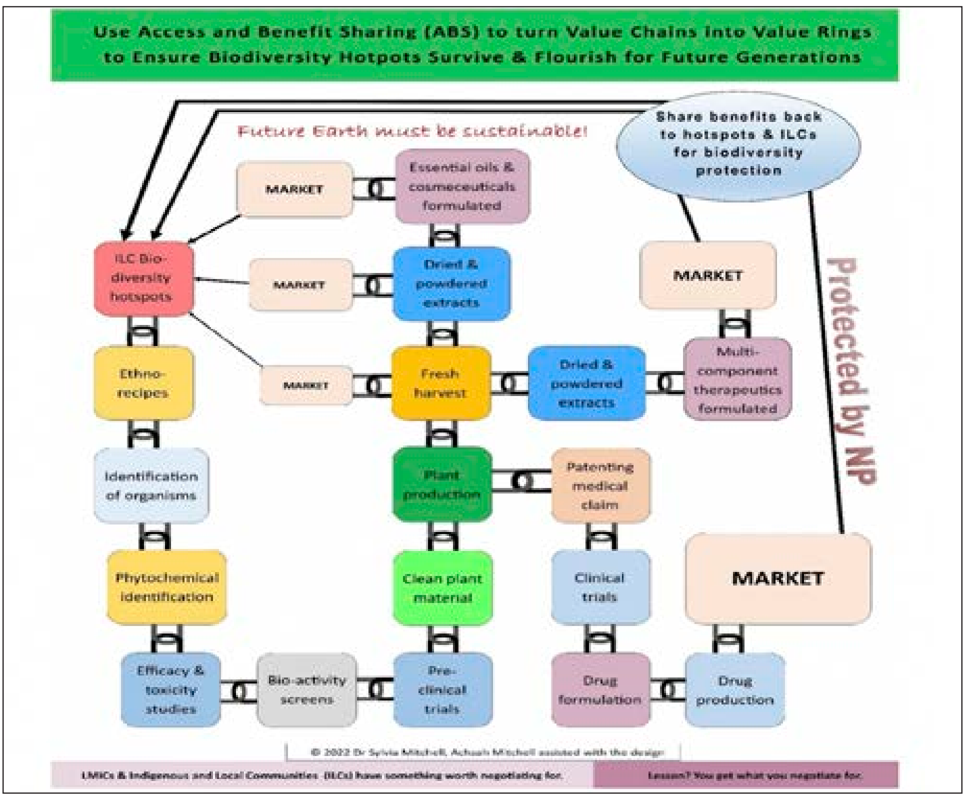

Figure 2: Value-chains turn to Value-rings in a Value-Web to support Sustainability of Genetic Resources and traditional knowledge in biodiversity-rich countries of origin

value-chains from biodiversity, for fresh produce, dried and powdered products (such as turmeric), essential oils and fine chemicals, nutraceuticals, cosmeceuticals (multi-component therapeutics) and pharmaceutical drugs. Modelling the value chains in a value-web, makes it easier to visualize the relationships of the market to the GR, TKs and ILCs, and their relationship to the Access and Benefit Sharing (ABS) envisaged by the Nagoya Protocol.

Opportunity 1: Become NP compliant, Ratify NP, and use the Nagoya Protocol

By overcoming the challenges outlined above and others, the remaining countries of the Caribbean will ratify the Nagoya Protocol and enjoy its benefits. Based on the bioprospecting cases shared in this paper, there is a long way to go to realize this opportunity. The Caribbean countries that have not yet ratified the Nagoya Protocol (Table 1) still require help to do so. This paper is written in support of CARICOM coming together and ratifying the Nagoya Protocol as one avenue towards redress of the historical problem of biopiracy as part of the inequitable, extractive, biased, racist colonial plantationocene. Suggestions of how to turn these challenges into opportunities is given in Table 6.

Table 6: How to turn challenges into opportunities to become NP compliant

Challenges	Opportunities
Too little action, inertia	CARICOM and regional universities should OWN and LEAD the process since most countries still to ratify are in CARICOM
Continuity is lacking, too few meetings, officers keep changing especially due to external factors such as COVID	Develop a CBD-NP-ABS bioprospecting website hosted by CARICOM and accessible to ILCs and regional universities, inviting every country in the Caribbean to take part; Rotate leadership amongst countries
Biopiracy is still happening	Develop a reporting system so what happens in one country can be known by other countries; establish a standardized bioprospecting registry
Information on completed and ongoing relevant projects (GIZ, GEF, UNEP, IUCN and others) not easily available	Establish a database of past regional ABS projects and outputs and make it available to all Caribbean countries via the proposed CBD-NP-ABS website. OWN the process!

Table 6 continues

Table 6: How to turn challenges into opportunities to become NP compliant *(cont'd)*

Challenges	Opportunities
Patents do not include country of origin of GR nor ILCs	Patents should be required to declare the country of origin, any involved ILCs (e.g., where ethnobotany surveys were conducted) and any local participants involved. Where not possible a competent authority be established to issue internationally-recognized certificate of compliance prior to acceptance of a patent request.
Patents do not recognize communal knowledge	Develop better forms of ILC rights recognition that are grounded in indigenous systems of authority and knowledge, reflective of communal and ecological systems of management of biocultural resources.
There are no Caribbean-wide standard MAT and PIC forms	Standardize forms at least for CARICOM and put them on the website in all languages of the Caribbean
As well as benefit sharing, more research is needed for GR & TK	This requires sustained attention to our Flora and Fauna from government, academia, ILCs and private sector.

Opportunity 2: Stabilise the bioprospecting value-chain by using the principles of Ecobioprospecting and Ethnobiotechnology

Countries of origin are biodiversity-rich tropical countries and unlike User countries, need to put more attention on the earlier links in the value-chains (Figure 2) The NP aims to return benefits many years after the original biodiversity was accessed. The ABS system in countries accessing external GR does not need to include procedures to ensure sustainability of the GR accessed. However, those countries from which GRs are being accessed (usually Biodiversity Hotspots) need to ensure their GRs are sustainably maintained. Ecopharmacognosy was coined by Cordell (2013) to describe the "study of sustainable, biologically active natural resources" to ensure that 'selected, valuable traditional medicines are not depleted in their natural environment, but instead alternative resourcing is developed'. This paper recommends applying the principles of Ecopharmacognosy.

Due to the historically extractive plantationocene period, with ongoing neglect of the country of origin (due to non-sharing of benefits), in this case the Caribbean Island Biodiversity Hotspot, and notwithstanding the efforts of international organizations such as UNEP, CEPF and IUCN, sustainability of the bioresource is still threatened (Mitchell et al., 2018a, 2019). There remains a need for plant pictures in regional floras, morphological and ecological studies, and support for regional herbariums including putting them online. There remains need for basic botanical research to identify medicinal plants and their bioactive phytochemicals that are known only by ILCs for example, the endemic root tonic plants in Jamaica (Mitchell, 2011). This is where ethnobiotechnology can help.

Ethnobiotechnology is concerned with developing biotools and biotechniques that are tailored to and appropriate for indigenous people and their communal knowledge systems, and local communities of the small, developing, tropical states of the Caribbean (SIDS) (Mitchell, 2014). Ethnobiotechnology is the means whereby we can achieve ecopharmacognosy. Studying plants, or any natural organism, for their use as global medicine, must take into account proper identification and long-term sourcing. Support is needed to: identify organisms (morphology, genetic fingerprint, common names, scientific names, pictures of their life cycle stages, chemical fingerprint, etc.), monograph development, adequate and accessible regional flora and fauna guides, online access to regional herbariums, propagation and conservation protocols, and ethno-appropriate school gardens and curriculum. Any associated folk knowledge of how biodiversity can be used, whether for health, to weave baskets, feed animals and so on, should be recognized as intellectual property of the ILC and a method devised to recognize this Intellectual Property Right (IPR). This paper recommends developing ethnobiotechnology solutions.

Ethnobiotechnology can help to support this process by

- Ethnobotanical studies
- Development of local community in vivo, ex vivo and in vitro germplasm gene banks (for conservation)
- Development monographs of folk plants including production of genetic fingerprint, chemical fingerprinting, bioactivity screens and morphological studies.
- Efficacy and toxicity studies of ethnorecipes (Ethnopharmacology)
- Clonal multiplication of chosen folk plant germplasm via tissue culture
- Screening of phytochemicals via tissue culture
- Production of high-value ethnobiopharmaceuticals via tissue culture
- Product development of essential oils and fine chemicals linked to on-farm testing of quantity and quality of yields supporting local industries, including ILCs.
- Using biotechnology to put life back into our soils (biosoils) by using micro-

bial inoculants, biochar etc linked to certified disease-free micropropagated plants, field trials and post-harvest studies.

- Producing ethno-compliant bio-active drugs, nutraceuticals, cosmeceuticals, and multi-component therapeutics.

Opportunity 3: Turning Value-chains into Value-rings for bioresource survival

Not all the links in the value-chains depicted in Figure 2 are usually recognized in the typical bioprospecting value-chain that only considers four phases: collection, identification, screening and commercialization (Melgarejo and Química, 2013; Aquaculture, 2022). A value-chain, which shows how each stage fits into the other, all the way from the bio-resource to various markets is more appropriate to visualize how benefits should be shared (Figure 2). Benefit sharing mechanisms that result in fair and equitable distribution among all parties concerned, including local communities, indigenous groups, universities, companies, countries should be the goal.

Forecasting the future allows one to imagine what a value-web needs to include. When the market is freshly harvested herbs, funds easily reach back to the community that grew them. This then is a value-ring. As the plant material gets dried, powdered, extracted, the value-chain gets longer, and rewards increase while the source is increasingly ignored. Mechanisms such as Fair Trade have been enacted to ensure a value-ring for food crops such as cocoa. However, the longest chain (pharmaceutical) has the most links and the most reward. The Nagoya Protocol attempts to turn this into a value-ring and while it works for drugs in some cases, it is not as easy for other products made from medicinal plants nor for associated traditional knowledge that is held communally in ILCs.

Opportunity 4: Use the principles of the NP where it does not apply

Is the Nagoya Protocol (NP) the only way the Caribbean can obtain benefits from use of its GR and TK, for conservation and developmental purposes? Not at all. The Nagoya Protocol does not apply to multi-component therapeutics, nutraceutical, functional foods or cosmeceuticals made from plants such as pimento to make Jerk Sauce, Khus Khus to make salves, bay leaves to make bay rum. However, the principles of the ABS of the NP with PIC and MATs can still be utilized. Also, other mechanisms and types of IP protection may be applied to cover these products so that the biodiversity hotspots can be sustainably used and maintained e.g. geographical indicators[7].

Is Access the best criteria on which to base Benefit Sharing? The NP depends on compliance certificates linked to the patent system to link Access to Benefit Sharing, especially between countries. The patent system does not acknowledge communal knowledge. Therefore, the TK of indigenous groups needs a form of rights recognition. Other mechanisms that can be used to protect these inventors include copyright, trade secrets, geographical indicators, copyleft, and restricted databases.

Opportunity 5: Develop a regional and standardized bioprospecting database

While each country ratifies the NP, there is an opportunity, and possibly a necessity to develop a regional ABS database to include: bioprospecting access requests and reports, Caribbean biodiversity-related patents, compliance certificates, ethics proposals from regional and extra-regional scientists, relevant Caribbean literature and reporting of derived products. Together, the Caribbean people must find solutions that will conserve biodiversity while allowing for sustainable use of this shared CIBH bioresource. An output of the UNEP project was the development of standardized methodologies that can be used for developing shared Caribbean national biodiversity registries (Mitchell et al., 2018d). Mechanisms for accessing these registries and the regional ABS database and for sharing benefits amongst the Caribbean nations should be developed. It should be hosted by the Caribbean for use by Caribbean nations.

Bioprospecting from the sea needs special considerations. This is because bioresources more easily move around and can thus be present in the seas of more than one nation. However, the Caribbean Sea has plenty of marine genetic resources to share. The value of products derived from marine genetic resources alone has been valued at $50 billion while a single enzyme isolated from a deep-sea hydrothermal vent used in ethanol production had an annual economic impact of $150 million[8].

7. See https://www.myersfletcher.com/resources/item/you-can-t-call-it-that-it-doesn-t-come-from-here-geographical-indication-protection-in-jamaica.html
8. See (https://dsmobserver.com/2020/07/bioprospecting-in-practice-how-a-drug-goes-from-the-ocean-to-the-clinic/)

Conclusion

We have found no comprehensive review of Caribbean bioprospecting nor corresponding investigations into biopiracy. Together with Mitchell et al (2022), this paper attempts to lay a foundation for continued analysis of Caribbean bioprospecting and its potential to enhance the region's development whilst supporting conservation of its biodiversity. Analysis of historic and more recent bioprospecting cases, and any associated benefit-sharing, produced as many questions as answers. Given that the Caribbean is the seventh largest biodiversity hotspot and has been subjected to a history of extractive bioprospecting (biopiracy), which is unfortunately still occurring, several challenges facing the region as it attempts to right this wrong have been presented along with five opportunities. It is hoped that this paper may nudge the Caribbean, and especially CARICOM, to ratify the Nagoya Protocol and establish a robust system to give access to its resources while using the shared benefits to ensure the CIBH remains vibrant, and the Caribbean countries develop sustainably.

Forecast For the Future

It is hoped that the outputs of the projects from 2012 to 2019 assist the remaining Caribbean countries to ratify the Nagoya Protocol and for all to use it so as to reap benefits for our region, for conservation and for developmental purposes. It is hoped that this paper helps clarify the issues and keeps the conversation going and encourages investments into knowing and valuing Caribbean biodiversity, for all our sakes. It is hoped that the Caribbean will develop value-rings and then use the benefits secured to locally develop more links in these biodiversity-driven innovative value-added-chains. It is also hoped that the processes put in place to activate access and benefit sharing within and between countries, will protect the indigenous peoples and help local communities to prosper. It is also hoped that where the Nagoya Protocol does not apply, that other mechanisms to protect our biodiversity for future generations will be developed.

Acknowledgments

We thank IUCN for this consultancy, UNEP for initiating this project, and GEF for funding. We especially thank Melesha Banhan as the IUCN project director for her guidance and insight during this project. We also thank all the peoples of the Caribbean that answered questionnaires, filled forms, attended the workshops and otherwise provided feedback. I also thank Drs Nicole Plummer, Patricia Northover, Charmaine McKenzie and Marisa Wilson for their comments on previous versions of this manuscript.

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The Journal of Arts Science and Technology

Preliminary Evaluation of Hygienic Practices of Street Food Vendors in Barbados

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Abstract

Foodborne diseases are often associated with poor personal and food hygiene of street food vendors. Street vended foods are Ready to Eat (RTE) meals prepared and served in public places. The main objectives of this study were to evaluate the hygienic practices of street food vendors in Barbados regarding the prevention of food contamination using a survey instrument and to assess of the microbiological quality of black pudding, pickled souse, and natural fresh juice as examples of RTE street foods over a six-week period. A total of 18 street food samples (6 samples each of pudding, souse, and natural juices) were purchased from street food vendors and analysed for coliforms, Escherichia coli, Staphylococcus aureus and Salmonella. The results of the surveys indicated poor sanitary levels in personal hygiene, food hygiene and a lack of food safety training were key indicators among street food vendors in general. Additionally, coliforms and E. coli were identified in the pudding and souse samples at unacceptable levels of contamination. The natural juice samples showed no microbiological contamination. The prevalence of coliforms was 72% and the prevalence of E. coli was 56% respectively of the analysed samples. Both S. aureus and Salmonella spp. were absent among the street food samples analysed. In conclusion, improvements are required on the hygienic practices of street food vendors in Barbados. Interventions, monitoring and the implementation of the HACCP approach should be regulated to maintain good hygienic practices and subsequently good microbiological quality of street food.

Keywords: Food Hygiene, Street Food Vendors, Personal Hygiene and Practices, Food Borne Diseases, Food Safety Knowledge, RTE

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Introduction

Street vended foods or "street foods" are ready-to-eat (RTE) meals prepared and served along the streets or in public places from stalls or kiosks. The World Health Organization (WHO) groups street foods into categories based on where the food is prepared: food prepared in small scale factories, food prepared at home, food prepared in markets and food prepared on the street (Umar et al., 2019). Street foods are usually consumed by a large segment of the population due to its convenience (inexpensive and easily accessible). Additionally, street food vending provides the resources for many in different socioeconomic classes and creates employment opportunities (World Health Organization, 1996). However, street food poses a major public health burden for consumers. Street food is recognized as a potential carrier of food pathogens which results in foodborne diseases. Foodborne diseases are defined as diseases or illnesses caused by contaminated foods and such foods can become contaminated through the means of bacteria, viruses, parasites, or toxins (Qadir et al., 2017). This growing concern of food hygiene among street food vendors is mainly due to the issue of a lack of food and personal hygiene; and/or the sanitary conditions of vending sites.

Food handlers' hygienic practices are the predominant means of transmission of foodborne diseases among street food vendors and a major source of microbial contamination in foodborne illness outbreaks is through human contact. According to Guzewich and Ross (1999), 89% of outbreaks in the United States of America (USA) yearly were caused by transmission of pathogens by food handlers. Pathogens are potentially transmitted to food when food handlers touch contaminated surfaces and/or contaminated food combined with improper handwashing practices.

Another leading problem that contributes to foodborne diseases from street food is the lack of food hygiene/ food safety awareness and implementation among street food vendors. This results in unsanitary practices such as improper holding temperatures of food and improper raw material management (Guzewich and Ross, 1999). The lack of food safety knowledge may be partly due to the lack of formal and informal food training. In developing countries, inadequate supervision, and a lack of monitoring by public health officials often results in poor implementation of food hygiene regulations/standards among street food vendors (World Health Organization, 1996).

One of the objectives of this study was to review the different aspects that contribute to the hygiene practices by street food vendors using a survey instrument. It was argued that the hygienic practices can be greatly improved once each contributory factor associated with street food vendors having a high-risk of contaminating food is held to Hazard Analysis Critical Control Points (HACCP) standards and with food safety training programmes implemented. With further research and added knowledge from studies such as this one, the hygienic practices of street food vendors as a means of preventing food contamination can be optimized. Another research objective was to examine the microbiological quality of RTE street foods in Barbados as an indication of the hygienic practices of street food vendors. It was hypothesized that if street food vendors operate with acceptable hygienic practices compliant to HACCP standards, the microbiological quality of street vended food will be acceptable for human consumption. This study focused on the microbiological quality of natural fruit juices, black pudding, and pickled souse as examples of popular street food.

Methodology

Survey

Street food vendors (N=30) were surveyed concerning the practices, knowledge and perception of street food and street food vending in Barbados. The street food vendors involved were in popular street food vending locations within the parishes of St. Michael and Christ Church as shown in Figure 1. A total of 30 street food vendors participated for this survey. The 15-minute self-administered survey consisted of *yes* and *no* questions and open-ended questions.

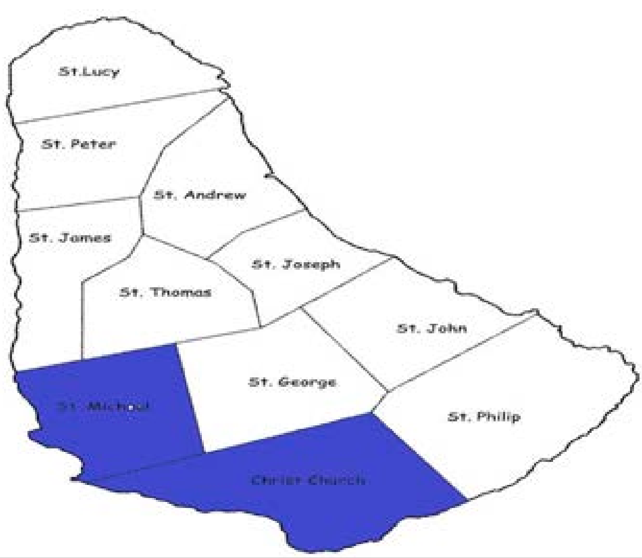

Figure 1: Map of Barbados showing Location of Research Population.

Sample Collection and Analysis

A sample of pickled souse, black pudding and natural fruit juice were collected from street food handlers and aseptically transferred to the laboratory for same day analysis. Each sample was analysed for total coliforms, *E. coli*, *S. aureus* and *Salmonella* spp. This procedure was repeated weekly for six weeks (Table 1). The samples were analysed using the procedures found in the U.S. Food and Drug Administration *Bacteriological Analytical Manual* (8th edition) for food samples.

Table 1: Lab Frequency and Methodology Summary

Lab Session	Sample Collection	No. of Samples to be tested	Tests
Week 1	Location 1- Samples A & B Location 2- Sample C	3	Food Sampling Coliform testing *E. coli* testing *S. aureus* testing *Salmonella* testing
Week 2	Location 3- Samples A & B Location 4- Sample C	3	Food Sampling Coliform testing *E. coli* testing *S. aureus* testing *Salmonella* testing
Week 3	Location 5- Samples A & B Location 6- Sample C	3	Food Sampling Coliform testing *E. coli* testing *S. aureus* testing *Salmonella* testing
Week 4	Location 1- Samples A & B Location 2- Sample C	3	Food Sampling Coliform testing *E. coli* testing *S. aureus* testing *Salmonella* testing
Week 5	Location 3- Samples A & B Location 4- Sample C	3	Food Sampling Coliform testing *E. coli* testing *S. aureus* testing *Salmonella* testing
Week 6	Location 5- Samples A & B Location 6- Sample C	3	Food Sampling Coliform testing *E. coli* testing *S. aureus* testing *Salmonella* testing

Key: Sample A: Pickled Souse; Sample B: Black Pudding; Sample C: Natural Fruit Juice

Coliform and Escherichia Coli Testing

Twenty-five grams (25g) of sample A (souse) was weighed and added to a stomacher bag containing 25ml of diluent. The bag containing the mixture was placed in the stomacher for 1 minute at 230 rpm and stomached to achieve homogenization. The 50ml mixture was then added to 200ml of diluent to make a 250ml solution. A serial dilution was created using the 250ml sample solution and diluent as the first dilution (10^{-1}). To make the serial dilution, 10ml of the 250ml homogenized solution was measured using a 10ml glass pipette and added to 90ml diluent creating a 10^{-2} dilution. The 10^{-3} dilution was created using 10ml of the 10^{-2} dilution and adding to the 90ml diluent. After the serial dilution was created for Sample A, the process from weighing of the sample was repeated for Sample B (pudding) and Sample C (natural fruit juice).

From each dilution (10^{-1}, 10^{-2}, 10^{-3}), a 1ml inoculum was plated (in duplicate) onto *Escherichia coli* (EC) Petri-film plates. The plates were incubated for 24hr at 35°C. After a 24hr incubation, colonies with distinct appearance/colour were counted on the EC plates using a Petri-film reader machine. The colony count was recorded.

Staphylococcus Aureus Testing

After the serial dilution was created for Sample A, from each dilution, a 0.1ml inoculum was plated (in duplicate) onto Baird Parker plates and spread. The spread plates were incubated for 24hr at 35°C. After 24hr incubation, black colonies with halo were counted and presumed as *Staphylococcus* species. To confirm *S. aureus* colonies, suspected colonies were aseptically inoculated into 0.5ml BHIB media tubes and incubated for 24hr after which, 0.5ml of coagulase rabbit plasma was added using a syringe to the BHIB tubes. After a 4hr incubation, clot formation was assessed by turning the tubes upside down. If a complete clot formation occurred, the suspected colony was noted as *S. aureus*.

Salmonella Testing

Twenty-five grams (25g) of sample A (souse) was weighed and added to a stomacher bag containing 25ml of buffer phosphate water (BPW) broth. The bag containing the mixture was placed in the stomacher for 1 minute at 230 rpm and stomached to achieve homogenization. The 50ml mixture was then added to 200ml of BPW broth creating a 250ml solution. This process was repeated for Sample B and Sample C.

The mixtures were placed at 35°C for 24hr after which, 1ml and 0.1ml of the enriched BPW were transferred to Tetrathionate (TTH) and Rappaport-Vassiliadis (RV) broths respectively for each sample and incubated for 24hr at 40°C. The inoculated TTH and RV broths were then streaked onto both Xylose Lysine Deoxycholate (XLD) and MacConkey agar and incubated for 48hr at 35°C. After incubation, colonies with specific growth on each agar were counted.

Results And Discussion

Personal Hygiene Practices

A major contributory factor to food safety of street food vendors is personal hygiene which mainly consists of hand hygiene and wearing of personal protective equipment (PPE) such as gloves, nets, aprons, and masks. It also involves practices such as maintenance of nails and not wearing jewellery while food is prepared and served.

Hand Hygiene

Table 2 illustrates the activities performed by food handlers that require thorough handwashing. Proper hand hygiene is essential in the prevention of food contamination by food handlers. During the period between 1993–1997, poor hand hygiene was found to be the source of contamination in 27–38% of foodborne illness outbreaks (Green et al., 2007). Bare hand contact during food preparation and serving can be a major source of contamination due to the exposure to skin microflora (Guzewich and Ross, 1999) which is divided into resident and transient microflora. Resident microflora includes microorganisms naturally found on the skin and always present while transient microflora are microorganisms that temporarily colonize the skin through the direct skin to skin contact or indirect contact with contaminated surfaces. The latter are of concern due to their resistance to mechanical friction such as handwashing and thus their ability to be easily transmitted (Guzewich and Ross, 1999). However, one resident microorganism *Staphylococcus aureus* is a growing concern in the food industry due to its opportunistic properties. In addition to skin microflora, food handlers' hands can become contaminated with microorganisms such as *Escherichia coli*, *Clostridium perfringens*, *Shigella* and Hepatitis A virus through faecal-oral means and *Yersinia*, *Proteus*, *Campylobacter* and *Klebsiella* through animal sources

(Guzewich and Ross, 1999). Monteiro et al (2017) showed that some Brazilian street vendors had unsatisfactory levels of hand hygiene, whereby 31% of the vendors surveyed worked when sick with diarrhea, influenza and throat infections.

Proper hand hygiene of food handlers mainly depends on their handwashing practices which involve removal of microorganisms and pathogens from hands by means of mechanical friction. With the reduction of microorganisms on the food handlers' hands, it lowers the risk of transference between food handler and food, equipment, and other workers (Todd et al., 2010). Proper handwashing practices are shown in Table 2.

The importance of hand hygiene was illustrated by Qadir et al (2017) who surveyed street food vendors about their knowledge regarding personal hygiene. Most vendors had knowledge of personal hygiene practices, but most did not implement them into their personal hygiene regime. For example, most did not practice handwashing with soap and water before food handling and after touching their body; however, they did practice washing after toilet use and after handling raw food. A similar outcome was also reported from a study conducted by Demir (2019), where 43% of the street vendors studied did not have adequate knowledge regarding personal hygiene. Contradictory to most research, a study on street

Table 2: Activities performed by food handlers that require thorough handwashing.

When handwashing is recommended	Activity
Before these activities	Preparation of Food
	Putting on gloves
After these activities	Touching raw meats
	Using the bathroom
	Coughing or sneezing
	Eating or drinking
	Touching body parts such as nose
	Taking off gloves
	Handling dirty utensils, equipment, or clothing

vendors in Paris showed a 67% staff hygiene compliance especially in general hygiene, wearing gloves, wearing nets, handwashing, and maintenance of clean, short nails (Czarniecka-Skubina, Trafialek, Wiatrowski, & Gluchowski, 2018).

Like the study done in Paris (Czarniecka-Skubina, Trafialek, Wiatrowski, & Gluchowski, 2018), all participants in the Barbados study stated that hands were always washed during preparation and 77.8% stated that gloves were used as shown in Table 3. From the food vendor survey administered, the hand hygiene practices of street food vendors in Barbados were satisfactory in the prevention of cross-contamination.

Table 3: Food Knowledge and Practices of Street Food Vendors

Items	Percentage (%)	Percentage (%)
	Yes	No
Is the food prepared on vending site?	100%	0
Do you find time during peak service to wash hands between food preparation?	100%	0
Do you use gloves during food preparation and serving?	77.8%	22.2%
Are you aware that foodborne diseases are associated with the consumption of contaminated foods?	71%	29%

Awareness and Implementation of Personal Protective Equipment (PPE)

Another aspect of personal hygiene is the implementation of PPE, the most common being disposable gloves. Gloves are used as a secondary hurdle or barrier to control the transference of microorganisms from hands to food or from food to hands. Gloves are most effective when worn properly, consistently and changed frequently. Data from Figure 2 showed that gloves were changed often during food preparation and serving by 71.4% of food vendors, and sometimes changed by 29% of food vendors. The implementation of PPEs among street food vendors in Barbados, especially glove use, is of a satisfactory level and most likely contributed to a low possibility of cross contamination of food via street vendor to food route.

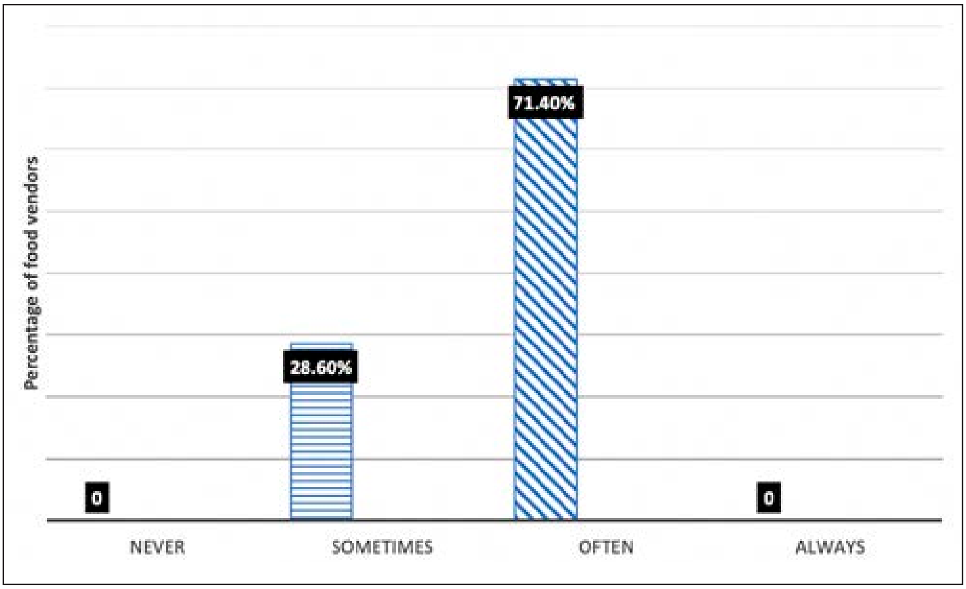

Figure 2: The frequency of glove changes among food vendors

Other PPE commonly used by food handlers are hair nets and aprons used to prevent bacterial transference from food handlers to food. However, the issue arises when street food vendors do not use and/or misuse PPE items particularly notable in developing countries. Adane et al (2018) explored safety measures among street vendors in Dessie town, Ethiopia and noted that most vendors knew the importance of PPE however only 37% used aprons. Additionally, the unhygienic practices of not wearing hair nets and smoking were seen among some street vendors in Kaduna State, Nigeria (Monteiro et al., 2017). Some street vendors lacked knowledge of personal hygiene which resulted in a lack of understanding about hygiene standards (Umar et al., 2019). Most studies indicated poor personal hygiene levels among street food vendors (Umar et al., 2019).

Sanitation of Utensils, Equipment and Working area

Kitchen utensils are tools used in food preparation, handling and serving, the common ones are spoons, forks, knives, and tongs. Similar, to glove use, utensils are used as a barrier method between food handlers and the food.

All utensils, equipment and food contact surfaces should prevent migration of substances which can harm the consumer upon consumption. Thus, these surfaces when in contact with food should not affect the colour, odour, or taste of the food. The US FDA (2017) recommends that food contact surfaces should be durable, non-absorbent, non-corrosive, smooth (free of cracks and pits) and easy to clean. These surfaces should be cleaned at least every four hours; before each use of a different type of raw material food; between touching raw foods and RTE foods or when contamination occurs (FDA, 2017). The cleaning frequency is dependent on the surface properties and use of utensils, the type of food, the amount of food residue accumulation over a scheduled use and the temperature at which food is being prepared and served.

Microorganisms can grow and survive on food residue (Dutra, 2017), for example food residues on stainless steel surfaces allow for the adhesion of bacterial biofilms. Hence if the utensils are of unsanitary conditions, potential pathogens can grow and be transmitted onto prepared food. While cleaning of utensils every 10-30 minutes falls under proper sanitation of utensils, this procedure was done only by 37% of Barbadian food vendors (Figure 3). Moreover, some street food vendors (13%) sanitized utensils every 5 hours which is of utmost concern since all street food were prepared on site (Table 3). These results indicated that utensils were being used continuously on a daily schedule with limited cleaning in between batches of foods. The transfer of pathogens and cross-contamination appears to be very likely in such a situation.

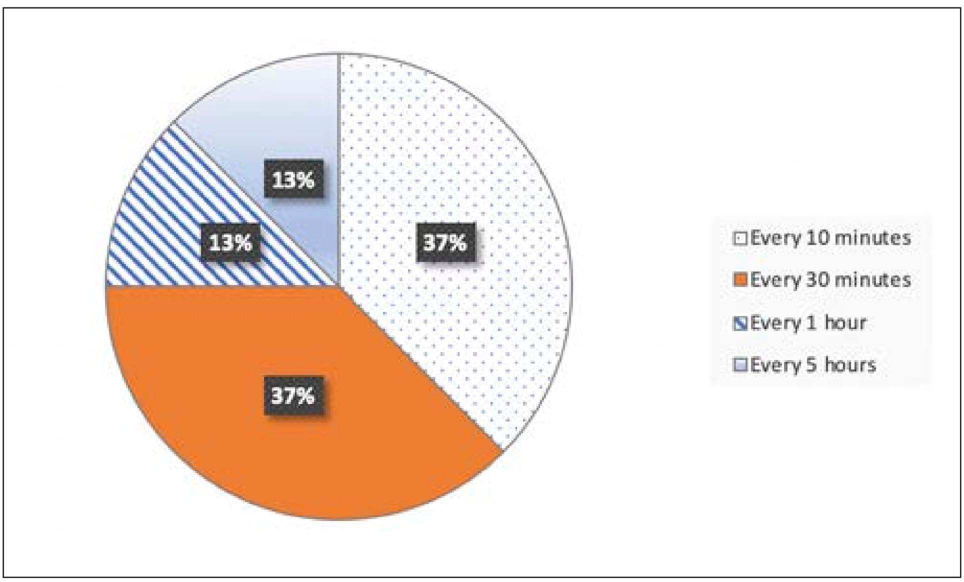

Figure 3: Frequency of cleaning and changing of utensils and equipment during food preparation among food vendors.

Food contact surfaces can be a potential source of food contamination whereby these surfaces allow for bacterial growth and survival if not properly cleaned and sanitized regularly (Scott and Bloomfield, 1990). Barro et al (2006) observed that bacteria such as *Staphylococcus aureus*, total coliforms and aerobic bacteria can adhere to utensil surfaces due to direct contact with food handlers' bare hands. Scott and Bloomfield (1990) documented that pathogenic bacterium, such as *Escherichia coli*, *S. aureus* and *Salmonella enterica* serovar Enteritidis, survive on surfaces such as hands and utensils for several hours after inoculation. These contaminated surfaces transferred significant bacterial counts onto food when in direct contact.

Zeru and Kumie (2007) investigated sanitary conditions of food establishments and its association with food contamination. Their study concluded that poor sanitary quality of food preparation utensils can arise from several factors such as ineffective washing and drying techniques and improper handling and storage. They also correlated poor sanitation in a food establishment to poor sanitary quality of the food preparation utensils. In these situations, the risk of food contamination due to dirty utensils is increased. Thus, the sanitation of utensils, equipment and working/preparation areas is highly important to the overall hygiene of the food served.

A study that focused on the sanitation of RTE food preparation areas was conducted by Little and Sagoo (2009). Upon examination, the cleanliness of the food preparation areas was unsatisfactory; chopping boards, food containers and worktop counter surface were contaminated with *E. coli* as these surfaces were cleaned with contaminated cloths and water (*E. coli*, coliforms and *S. aureus*). A higher risk of cross-contamination is presented due to the high inoculation levels on these food contact surfaces. Similarly, Monteiro et al (2017) reported that 40% of utensils and hands were contaminated with coliforms among Brazilian vendors. Street food vendors who clean their food preparation surfaces daily or even once a week pose a threat to food consumption due to potential bacterial growth and survival (Singh and Thakur, 2018).

Regular deep cleaning and sanitation with antiseptic agents reduces the bacterial load on utensils and working areas. Food contact surfaces should be cleaned after every meal and sanitized after every working day. Once street food vendors adopt proper sanitation regimes into their cleaning schedule, the potential of cross-contamination from food contact surfaces is greatly reduced.

Sanitation of Vending Sites

Vending sites polluted with garbage and/or located near stagnant water can attract insects and rodents leading to food contamination (Monteiro et al., 2017). Good sanitary conditions of vending sites may include, but not limited to, the presence of garbage bins, toilet facilities, wash sinks with soap and water and located away from dumpsters and areas free from the accumulation of dust and debris.

Several studies noted that many vending sites have relatively fair sanitary conditions (Umar et al., 2019). Most vending sites appeared cleaned with available waste bins for waste disposal, however, the presence of flies was seen at some vendors' stalls (Okajie and Isah, 2014). Singh and Thakur (2018) claimed that the cleanliness of the vending sites was mainly dependent on the street vendors; seeing that most sites were relatively clean indicated that most vendors cleaned their surroundings on a regular basis (presumably after every working day). From a public perception, the vending sites in Barbados had available bins and no stray animals but no toilet facilities with handwashing stations. The availability of waste disposal bins and absence of stray animals indicated good sanitary conditions but the absence of toilet facilities with no handwashing stations was a huge risk for cross-contamination in relation to hand hygiene.

Food Hygiene Practices

The main aim of food hygiene is the prevention of food contamination and subsequently foodborne illnesses and involves processes in food handling, preparation, and storage such as time/temperature control, storage temperature and raw material management. Good practices in these processes result in safe food for human consumption. Preparation of street foods should reduce and prevent food pathogens and hazards to an acceptable level for consumer consumption. In this step, it is critical to maintain food safety because it's the final step before sale. An essential rule in food preparation is to avoid direct and/or indirect contact between raw and cooked foods. Indirect contact occurs through means of contaminated hands, improper storage of raw materials and contaminated utensils or worktop areas. Street foods may be prepared through heat treatment (such as cooking, frying, or grilling) or other methods like fermentation/pickling. Ready-to-eat foods, such as, fruits and salads should be properly washed with clean water. After food preparation, storage time and temperature should be optimized to the specific street food to reduce growth of food pathogens.

Lack of food hygiene knowledge can develop into practices such as improper storage temperature before serving, lack of refrigeration, smoking in food preparation areas and talking over food (Monteiro et al, 2017; Qadir et al., 2017). Such practices introduce food pathogens and/or allow for their survival and growth which leads to foodborne diseases from street foods. Adane et al (2018) investigated food hygiene among street food vendors in Ethiopia and recorded that many vendors had good knowledge of hygiene practices and that food training and health education yielded higher levels of food hygiene.

Improper holding time during preparation was shown by food handlers in Barbados. Many participants stated that their food products were prepared/cooked in the morning before sales (Figure 4) however the peak vending time was in the evening/night (Figure 5). The time lapse between preparation and consumption meant cooked food was held at room temperature for extended periods of time exposing the food to microbial growth. Pudding and pickled souse are time sensitive RTE foods. Souse being held at room temperature and not chilled can be a reason for high microbial contamination. The holding time between preparation and serving can affect the microbiological quality of the food product. This time should be minimal to ensure optimal food temperature was kept. Ten to twenty (10–20) minutes between preparation and serving was executed by 40% of participants and less than 10 minutes by 32% as shown in Figure 6. According to Lakshman and Strohbehn (2013), the maximum of 4 hours holding time is crucial in proper food hygiene.

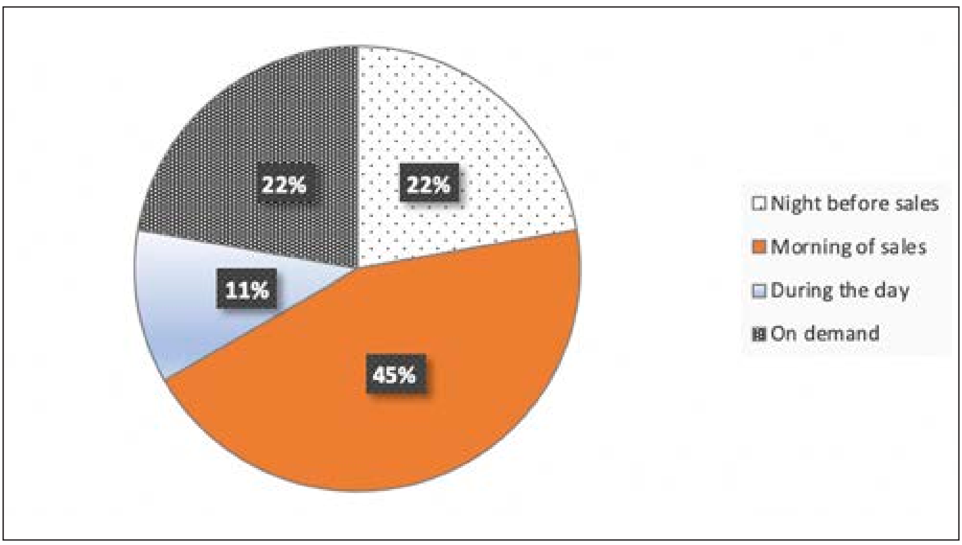

Figure 4: Designated time of food preparation before serving among food vendors

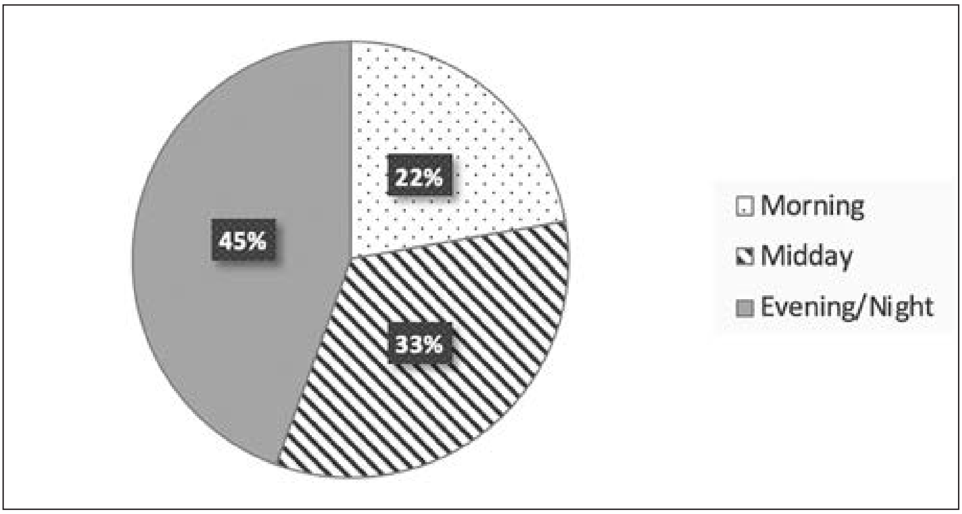

Figure 5: Peak vending time among food vendors

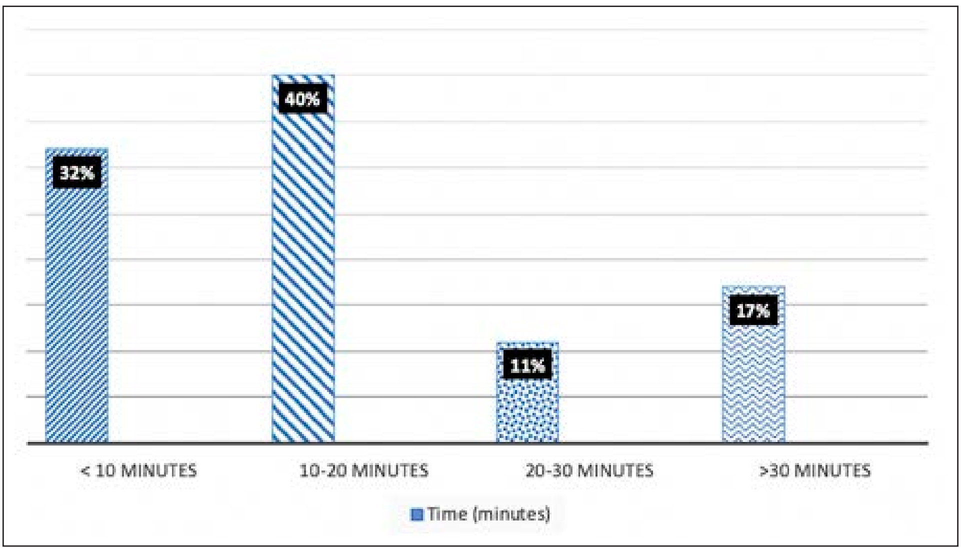

Figure 6: Time between preparation and serving among observed food vendors

Food Safety Knowledge

The food safety knowledge and attitude of food handlers is an essential factor in the prevention of food contamination. A general understanding of sanitary procedures can maximize safe food handling and preparation (Nee and Sani, 2011). Studies showed that educational training of food workers such as wearing of gloves yielded more hygienic food handling practices (Coleman and Roberts, 2005; Rajagopal and Strohbehn, 2013) and positive attitudes in food handlers resulted in more hygienic practices and workers becoming more accepting of educational training (Nee and Sani, 2011). However, a gap between food safety knowledge, formal training and hygiene practices has been observed among many street food vendors. Few street vendors have received formal training in food preparation and serving (Adane et al., 2018; Monteiro et al., 2017) and personal hygiene (Umar et al., 2019). Furthermore, some vendors lacked awareness of food safety laws and standards (Okajie and Isah, 2014). This lack of training and knowledge often leads to unsanitary practices among the majority of street food vendors.

However, knowledge of food safety does not always yield sanitary food safety practices. Training and knowledge of food safety practices sometimes are not sufficient for a food handler to practice sanitary activities (Nee and Sani, 2011). Rajagopal and Strohbehn (2013) found that the employees were involved in unsafe glove practices such not changing gloves after a specific task or after touching non-food items even though they were knowledgeable on glove use. Little and Sagoo (2009) also reported that the majority of street food vendors had food safety training, but only half of these complied with HACCP regulations.

Additionally, it was shown that age, working experience and educational status affected the attitudes of food handlers. A positive association between educational status and working experience and the implementation of sanitary practices has been exhibited (DEMIR, 2019). On the other hand, younger food handlers tended to be untrained and associated with food contamination (Monteiro et al., 2017). The gender of the food vendors however had no effect on the knowledge and attitude of food handlers.

Stated studies showed a positive association between food knowledge, training, and hygienic practices in food handling. Most food handlers in Barbados obtained food safety training or public health training as shown in Figure 7. Similarly, most of the food handlers (71%) were aware that contaminated food was linked to foodborne illness outbreaks (Table 3). It is commendable that many Barbadian food handlers obtained formal training and were knowledgeable in food safety practices.

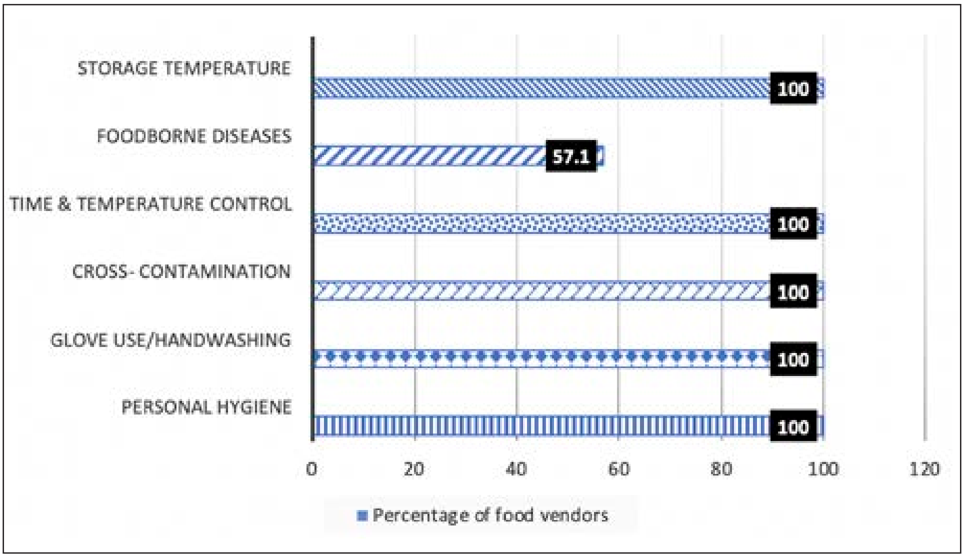

Figure 7: Food training obtained by street food vendors

Evaluation of Hygienic Practices of Street Food Vendors in Barbados

Microbial contamination of RTE foods is the main reason of foodborne disease outbreaks implicated by food handlers. According to Guzewich and Ross (1999), pathogens such as *E. coli* O157:H7, *Salmonella* spp. and *S. aureus* were responsible for approximately 12.3 million foodborne illness cases and 3,900 deaths yearly in the USA. Such studies are lacking in the Caribbean especially in Barbados where most of the foodborne illness cases from street foods are either under-reported or unreported. This section of the study focused on how the hygienic practices of street food vendors affected the finished quality of the pickled souse, black pudding, and natural fruit juice samples.

Figure 8 showed that over the 6-week study, pickled souse exhibited the highest total coliform count indicating that it was the most contaminated RTE food among the analysed samples. Coliforms were absent in the juice samples except for week 5. The prevalence of coliforms in all samples of pudding, pickled souse, and natural juice samples over the study period was 72% (13/18). However, the vast quantity of coliforms present in pudding and souse samples were concerning and it appeared that they were prepared, served and/or displayed with poor hygienic practices among the street food vendors.

Over the 6-week study as presented in Figure 9, pickled souse exhibited the highest *E. coli* count indicating that it was the most contaminated food sample

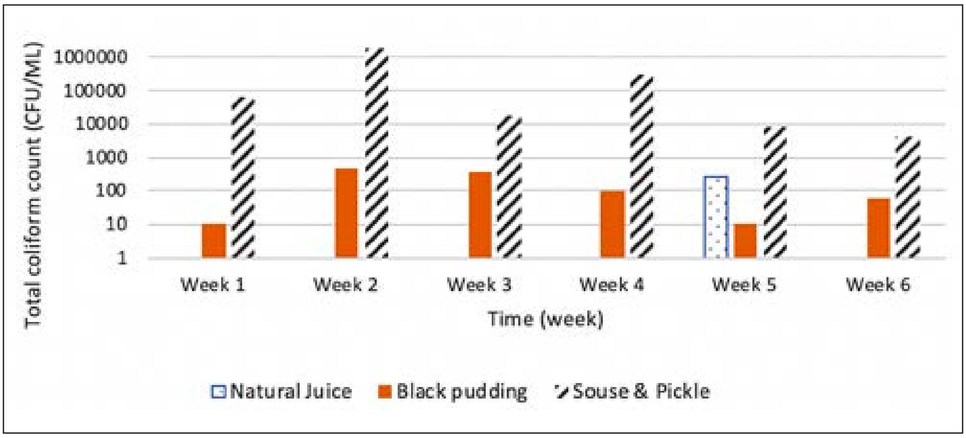

Figure 8: Total Coliform Count for natural fruit juice, black pudding, and pickled souse samples

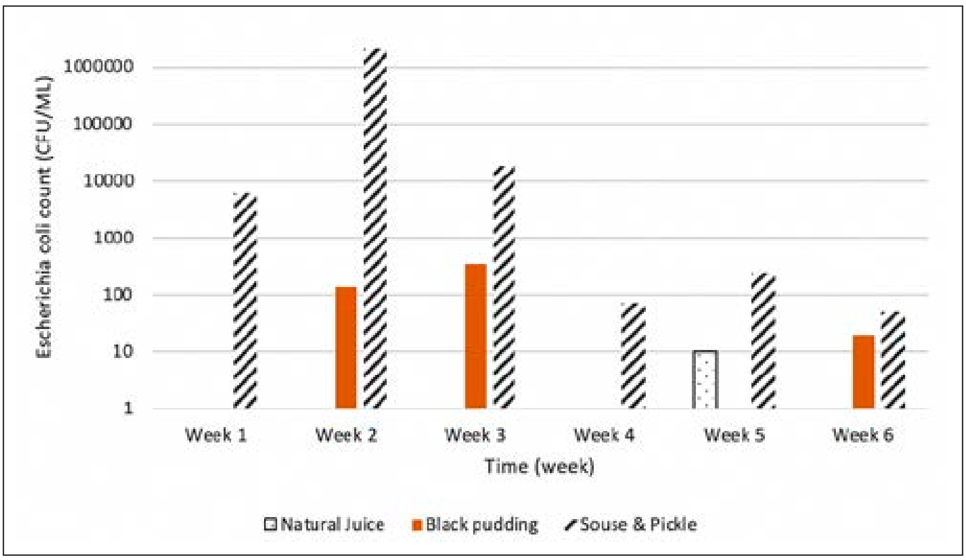

Figure 9: Escherichia coli count for natural juice, black pudding, and pickled souse samples

analysed. Like the total coliform count, natural juice samples showed no presence of *E. coli* except for week 5. The overall prevalence of *E. coli* in the pudding, pickled souse, and natural juice samples over the duration of the study was 56% (10/18). Similarly for coliforms, the identification of *E. coli* was mainly in the pudding and pickled souse. Unhygienic practices highlighted the prevalence of total coliform which can also be a means of *E. coli* cross-contamination of street food in Barbados.

Unlike coliforms and *E. coli*, the prevalence of *S. aureus* was 0% (0/18). Notably, *Staphylococcus* spp. was recorded in all food samples analysed however upon further testing (using confirmatory tests previously described), it was not confirmed as *S. aureus*. Most species of *Staphylococcus* are skin microflora. Therefore, even though *S. aureus* was not identified in the food samples, poor hand hygiene practices were still evident from this study.

Additionally, the prevalence of *Salmonella* spp. in the food samples analysed was 0% (0/18). The absence of *Salmonella* spp. indicated that the methods of food preparation among street food vendors were of satisfactory levels in the eradication of the *Salmonella* bacteria from the raw food materials.

Coliforms and *E. coli* were the main bacteria of concern in the assessment of contamination of street food. The main assumption for cross-contamination was poor hand hygiene practices among the street food vendors in Barbados. Even though most food handlers in Barbados received formal training and had food safety knowledge, the microbiological quality of the street food examined needed improvements. As noted prior, knowledge and training does not always yield more hygienic practices (Little and Sagoo, 2009). Food handlers can be knowledgeable in food safety but still practice unsanitary practices during food preparation and handling. Even though most of the food handlers had food safety training, the microbiological analyses of souse and pudding showed unsatisfactory levels of contamination. This demonstrated that the food handlers did not comply with the sanitary standards/HACCP regulations which was taught in food training. Overall, the hygienic practices of street food vendors in Barbados were deemed unsatisfactory with regard to HACCP compliance based on the poor microbiological quality of tested street food.

Conclusion

This paper reviewed personal hygiene, food hygiene, sanitation of utensils/equipment, sanitation of vending sites and food safety knowledge as major contributory factors to the hygienic practices of street food vendors. The findings indicated satisfactory hygienic levels in sanitation of utensils/equipment, sanitation of vending site, food knowledge and food training and unsatisfactory hygienic practices in personal hygiene and food hygiene. Based on the responses received from the food vendor survey, the leading issues among the street food vendors' hygienic practices were improper holding time and temperature of time sensitive RTE foods such as pudding and pickled souse which most likely elevated the bacterial growth.

While the food safety training and food knowledge among street food vendors were sufficient, it did not yield hygienic practices in all aspects of the evaluation.

The microbiological quality of RTE pudding and pickled souse samples showed unacceptable levels of *E. coli* and coliforms indicating poor sanitation and improper personal and food hygiene.

In general, improvements in the hygienic practices of street food vendors are greatly needed. Hand hygiene practices and food safety training programmes need to be implemented and standardized among street food vendors in Barbados to reduce food contamination risk. These improvements can be achieved through routine monitoring and compliance to food safety laws/regulations and HACCP standards.

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The Journal of Arts Science and Technology

Assessment of a Small-Scale Cacao (*Theobroma cacao* L.) Fermentation Method for Niche Marketing

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Abstract

The burgeoning of small gourmet chocolate boutiques worldwide has generated interest in niche marketing of cocoa[1] microlots, branded as (a) geographical indication (b) estate origin or (c) based on unique genetics. Over 90% of the cacao farms globally are small with an average size of 2–5 hectares and can benefit from this trend. Fermentation of cacao[2] is critical for expression of genetic flavour potential, cessation of germination and pulp removal. Typically, fermentation methods require ≥1000.0 kg of wet cacao beans. These quantities are difficult to achieve in small-holder farms. Therefore, small-scale vessel prototypes (modular, insulated, Styrobox- 5.0 kg (SB-5) and 30.0 kg (SB-30) capacity); were compared and the better prototype tested against large scale standard-Commercial Wooden Box (CWB) fermentation, with respect to progression of temperature, pH and external bean colour (L, a, b) during fermentation and sensory data of finished product. In the comparison of SB-5 versus SB-30, the latter displayed higher temperature peaks and temperature and pH progressions more consistent with effective fermentation and resulted in optimum expression of flavour. In the comparison CWB versus SB-30, the average temperature of fermentation mass exceeded 44.0°C in both, but was higher in CWB (47.2°C) than SB-30 (44.0°C). Average pH trends of testa and cotyledon during fermentation was similar for

1. Refers to the seeds of the cacao tree that have been fermented and dried and to the manufactured product (the powder sold for drinking or food manufacturing purposes).
2. Refers to the tree, the pods and the unfermented beans from the pods.

both CWB and SB-30. The cacao beans decreased in lightness (L) as fermentation progressed; at a faster rate in CWB than SB-30, but resulted in similar end values. SB-30 required slightly longer fermentation (7 days) compared to CWB (5 days) for optimum flavour expression. Flavour attributes were not significantly different between CWB than SB-30 at their respective optimum fermentation times, indicating that the SB-30 prototype can be used for small-scale fermentation.

Keywords: Cocoa, Styrofoam, Cocoa Quality and Sensory Assessment

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Introduction

Cacao is an understory tropical tree species with its centre of diversity in the Upper Amazon region of South America (Zarrillo et al. 2018; Bergmann 1969). Fermented and dried cocoa beans are used to produce a range of confectionary products and beverages of economic value (Afoakwa 2016). The burgeoning of small gourmet chocolate boutiques worldwide serves as a key driver for niche and ultra-niche marketing of cocoa branded as (a) geographical indication (b) estate origin or (c) based on unique genetics. Over 90% of the cacao farms globally, are small with an average size between 2 and 5 hectares and can benefit from supplying to gourmet boutiques.

Fermentation of cacao is critical for expression of genetic flavour potential, cessation of germination and pulp removal. Typically, fermentation methods require ≥1000.0 kg of wet cacao beans. These quantities are difficult to achieve in small-holder farms/niche marketing scenarios and therefore suitably adapted fermentation vessels are required.

Previous field-based studies focused primarily on overcoming the challenge of maintaining bulk-to-surface area ratio required to achieve temperatures ≥44.0^{0}C necessary to facilitate inhibition of bean germination and maintain the appropriate microbial succession (Griffiths, 1958; Van Hall, 1914). This was achieved through subsidiary heating (McDonald, 1935), preserving the heat generated during fermentation through greater insulation (Dewitt, 1952; Dewitt, 1951), or using the heat generated from large fermentation masses (Sukha, 2008; Clapperton et al., 1994). See Figure 1 for historical timeline of previous research on field- based small-scale fermentation.

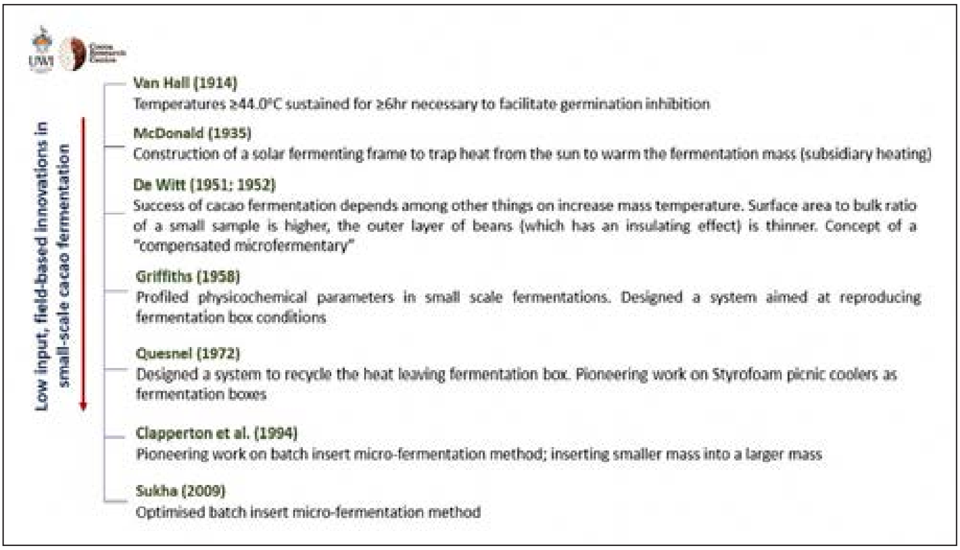

Figure 1. Historical timeline of previous research on field-based small-scale cacao fermentation

The fermentation vessel impacts surface to volume ratio, insulation and heat loss characteristics, drainage of sweatings and may also affect microfloral activity. The aim of this study was to assess a low cost, field-based, small-scale fermentation method capable of fermenting wet bean masses ≤30.0 kg, for use in niche marketing.The study was conducted in a two-tiered approach with the following objectives:

Phase I: small-scale vessel prototypes -modular, insulated, styroboxes 5.0 kg (SB-5) and 30.0 kg (SB-30) capacity were compared.

Phase II: the better prototype from Phase 1 was tested against standard large-scale wooden box fermentation in terms of development of temperature, pH, external bean colour (L, a, b) during fermentation and sensory data of resulting cocoa beans at the end of fermentation.

Methodology

Location and conditions

The fermentations were carried out at the Fermentation Facility of the Cocoa Research Centre, The University of the West Indies, St. Augustine Campus (CRC-

UWI). Cacao beans from three-quarter ripe pods (fruits) harvested from the estate at La Reunion, Trinidad was used in the study. La Reunion is located 15.0 m above sea level (10°34' N latitude and 61°18'W longitude), receives a total rainfall of 1870.0 mm and has an average temperature of 26.0°C (ClimaTemps 2018). Studies were conducted at start of the main harvesting season, (December to February) across two crop years. Wet beans extracted at the growing location were transported to the fermentation facility in clean polyethylene bags (80.0 cm (length) × 50.0 cm (width)).

Fermentation Vessels

Studies were conducted in (a) 17.0 cm (height) × 27.0 cm (length) × 26.0 cm (width) Styrofoam box with 3 drainage holes (1.5 cm diameter, arranged diagonally across base of box), containing 5.0 kg of wet cacao beans (designated 5.0 kg Styrobox or SB-5); (b) 32.0 cm (height) × 44.0 cm (length at base) × 28.0 cm (width at base) × 48.5 cm (length at top) × 31.5 cm (width at top) Styrofoam box with 6 drainage holes (1.5 cm diameter, arranged in a triangular mirror image across base of box) containing 30.0 kg of wet beans (designated 30.0 kg Styrobox or SB-30); (c) in three commercial sized wooden boxes 103.0 cm (height) x 152.0 cm (length) x 153.0 cm (width) arranged in a cascading manner, holding 2000.0 kg of wet beans (designated Commercial Wooden Box or CWB) with slatted base for drainage (0.5 cm-width between slats).

Turning (aeration/homogenisation)

For styroboxes, turning consisted of emptying the contents of the boxes into two clean plastic tubs, the top half in one and the bottom half in another. Beans in each tub were mixed thoroughly with a clean plastic scoop to ensure aeration, homogenisation and distribution and returned to the Styrobox in a reverse manner (bottom of box filled with beans previously at the top and top filled with beans previously at the bottom). In the case of the CWB, fermenting beans at the top of the box were mixed with a wooden shovel and pushed from the top of first box into the bottom of the consecutive cascading box. Then the bottom half of the beans were thoroughly mixed with the shovel and pushed into the top of consecutive cascading box, as per commercial best practise.

Sampling

For all fermentation boxes, zones comprising- top left corner (1); middle- centre of box (2); bottom right corner (3); were used for sampling and data collection. Zone 1 was closest to wall of building and zone 3 was closest to corridor in front of box.

Experiment set-up

Phase I

Wet beans were derived from standard genetic groups, Amelonado and Refractario and used for the study. These were each subjected to fermentation side-by-side in two sizes of Styrofoam boxes, (a) 30.0 kg (SB-30) and (b) 5.0 kg (SB-5). The treatments (cacao types and fermentation box size) were arranged in a 2 × 2 factorial design, with two replications. Fermentation progression was monitored viz. temperature and pH measurements and final quality assessed by sensory analysis. Duration of fermentation spanned 0–7 days with turnings on days 3 and 5.

Phase II

Simultaneous fermentations of a defined mix of Trinidad Selected Hybrids (TSHs) were carried out in (a) Commercial Wooden Box (CWB) (cascade design, standard- 1000.0 kg) and (b) 30.0 kg Styrofoam box (SB-30). Temperature readings were taken daily and samples for pH, bean colour and sensory profiling (Ali 2022; Sukha 2008) were collected from each zone at the same depth (12.0 cm) every two days and composited for assessments or further processing. Duration of fermentation spanned 0–7 days with turns on days 2 and 4.

Data Collection and Analyses

Temperature readings were taken at the same depth (16.0 cm) in the three zones of the boxes, daily (Digi-Sense 94460-40 thermometer). To determine pH of testa and cotyledon, 9 beans selected from each composite sample were cut open, testae and cotyledons separated, placed into groups of three (three replicates), macerated in ceramic mortars and pestles (Coors USA 60313) with 10.0 ml deionised water. pH recorded using an Oakton® Acorn pH meter (Model no. WD-35613-70; USA). Similarly, pH of the fermentation mass was recorded from three zones (depth: 12.0 cm) daily (Oakton® Waterproof pH Spear 35634-40; USA) and averaged to get daily values. Samples for physicochemical and sensory profiling were collected from each zone at the same depth (12.0 cm) every two days and composited for testing.

External bean colour of fermenting beans (days 0, 2, 5, 7) was determined for treatments (SB-30 versus CWB only) as follows; beans were collected randomly (from composite sample) on specified days, placed into petri dish and four consistent spots scanned for L (lightness), a (-green, +red), b (-blue, +yellow) scores using a colorimeter (Konica Minolta, Chroma Meter CR-410 A 840 3057, Konica Minolta Sensing Inc., Japan) and averaged to obtain values over the time course. ereeach petridish,termined by ed into four for a paper. did not discover this.

Bean Drying Protocol

Sun-drying consisted of spreading beans on wooden, divided, slatted drying trays (depth 5.3 cm; 1.0 cm spaces between slats). The beans were arranged in ridges (3.0 cm high) and furrows (4.0 cm wide). The beans were turned every 2 hours and placed back into the ridge and furrow layout. Average daytime temperatures during sun-drying (January–March) fell in the range of 32.0–36.0 °C (Mujaffar, Sukha, and Ramroop, 2018). The beans were gradually dried, as per best practice, receiving 2 hrs of sun exposure on day 1 of drying, with increasing exposure time as days progressed. Typically, with good sunshine the beans dried within 4–5 days.

Bean Moisture Content

To determine the drying end-point, a digital moisture meter (Burrows Digital Moisture Computer; Model 700; Serial No. 24542; Seedburo Equipment Company, Chicago, Illinois) was used. All samples were dried to an average moisture content within the range of 6.0–7.0% (End and Dand 2015).

Cocoa Liquor Preparation

As part of the sensory assessment, for each sample, plump, undamaged beans were weighed (RADWAG USA LLC; Model: WLC 20.X2; Serial No. 506638, Poland) and spread to single bean thickness (1.0 cm) on stainless steel roasting trays 37.5 cm (length) × 22.5 cm (width) × 5.0 cm (depth); mesh 0.6 cm × 0.6 cm; capacity 330.0 g) and roasted at 140.0 ^{0}C for 30 minutes (Shel Lab SL Oven (Model no: 1350FX; Sheldon Manufacturers Ltd. OR USA). After roasting, beans were cooled, broken (John Gordon, Maldon Essex, England; serial number: LH-29/5816) and winnowed (John Gordon, Maldon Essex, England; serial number: CCI-55/5816).

The resulting nibs were coarsely ground (Cuisinart coffee mill, Model DCG-12BC (FA) Serial 108 28 P, East Windsor NJ 08520) and converted to liquor (paste) via grinding for 90 min in an end runner mill (Pascal Engineering Co Ltd. Crawley Sussex, England; Machine no: 23615) at a temperature of 60.0–70.0°C, utilising a heat gun (Black and Decker, HG 1500-B3, US Inc. Hunt Valley MD 21030) to a final particle size between 14–25 microns (Sukha et al. 2008). The liquor sample was transferred to labelled 4.0 oz sterile specimen containers, (VWR International, USA) and aged in storage freezer (-2.0°C) for a minimum of two weeks before sensory assessment.

Sensory assessment of cocoa liquor samples

Coded, randomised samples with two replicates were assessed by a trained panel of six using the protocol and sensory evaluation template of ESSeguine-DASukha Cocoa and Chocolate Flavour Evaluation by E.S. Seguine and D.A. Sukha licensed under a Creative Commons Attribution-ShareAlike 4.0 International License.

Data Analysis

The analysis was carried out using the software package Minitab 17 (2016), Minitab Inc., State College, Pa., USA or NCSS Version 07.1.19, Hintze, J. (2009), NCSS, LLC, Kaysville, Utah, USA.

Results

In Phase 1 of study, both SB-30 and SB-5 reached temperature peaks on day 4 of fermentation (Figure 2). Temperature trends revealed that both the SB-5 and SB-30 fermentations crossed 44.0°C, however SB-30 fermentations displayed overall higher temperature peaks. In the case of SB-30 two temperature peaks were observed after the first (Day 3) and second (Day 5) turnings (Figure 2), both peaks were close to 50.0°C. In the case of SB-5 a peak temperature of 45.0°C was observed after the first turning but no additional peak following the second turning. Average pH trends (mass-beans/pulp in fermentation box) showed that by day 6 the SB-5 fermentations had very a high average pH, while that for SB-30 was around 4.0 (Figure 2).

With regards to genetic groups differences, Amelonado took longer to reach temperature peak and exhibited variability in time to pH intersection. Both

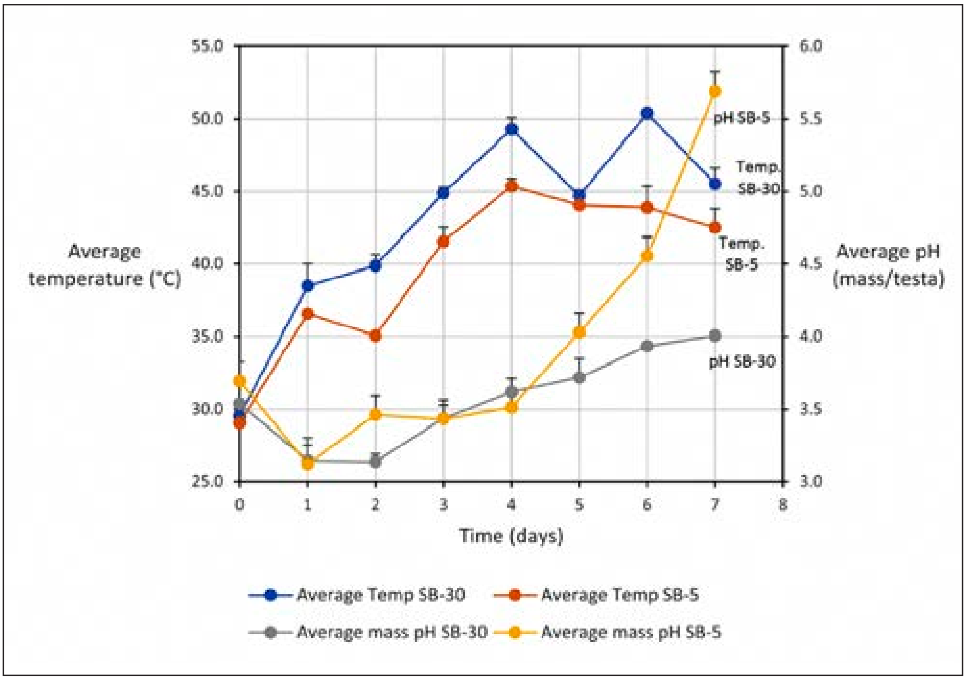

Figure 2: Comparison of average temperature and pH (mass) readings as fermentation progressed for SB-5 and SB-30 cacao fermentation prototypes.

groups exhibited varying graphical trends but were not significantly different ($P \geq 0.05$) in terms of temperature or pH progression.

Flavour attributes of the two genetic groups were not significantly different ($P \geq 0.05$) and there was no genetic group × treatment interaction. However, there were significant differences in terms of fermentation vessel size (treatment) for the following flavour attributes; acidity ($P \leq 0.05$), astringency ($P \leq 0.001$), bitterness ($P \leq 0.001$), and fresh fruit ($P \leq 0.001$) (Table 1). The average sensory attribute scores for acid, astringent, bitter, fresh fruit were higher for SB-30.

In Phase II, average temperature across the three zones within the 30.0 kg Styrofoam box (SB-30) were overall significantly lower ($P \leq 0.05$) than corresponding temperatures in the traditional large commercial wooden box (CWB) but exhibited a similar graphical trend (Figure 3). Average temperatures exceeded 44.0^{0}C in both boxes by day 3 following the initial turning on day 2, albeit the peak temperatures were different, higher in CWB (47.2^{0}C) than SB-30 (44.0^{0}C). Similarly, following the second turning at day 5, temperatures peaked again in both CWB and SB-30; analogously, the peak temperature was about 3.0^{0}C higher in CWB than in SB-30.

Table 1: Summary of significant effects across flavour attributes for sensory samples generated for comparison of SB-5 and SB-30 cacao fermentation prototypes.

Factor	Replicate		Treatment (Fermentation Size)		Treatment × Day	
	Sig.	F value	Sig.	F value	Sig.	F value
Cocoa	NS	1.22	NS	0.14	NS	0.05
Acidity	NS	0.80	*	4.60	NS	0.51
Astringency	NS	0.70	**	7.12	NS	0.11
Bitterness	NS	1.69	**	6.26	NS	0.25
Fresh fruit	NS	0.63	***	13.97	NS	3.03
Browned fruit	NS	0.16	NS	0.45	NS	0.31
Floral	NS	0.38	NS	2.41	NS	0.12

NS - Not significant at (P>0.05). *, **, *** refer to significance at P≤0.05, P≤0.01, P≤0.001 respectively.

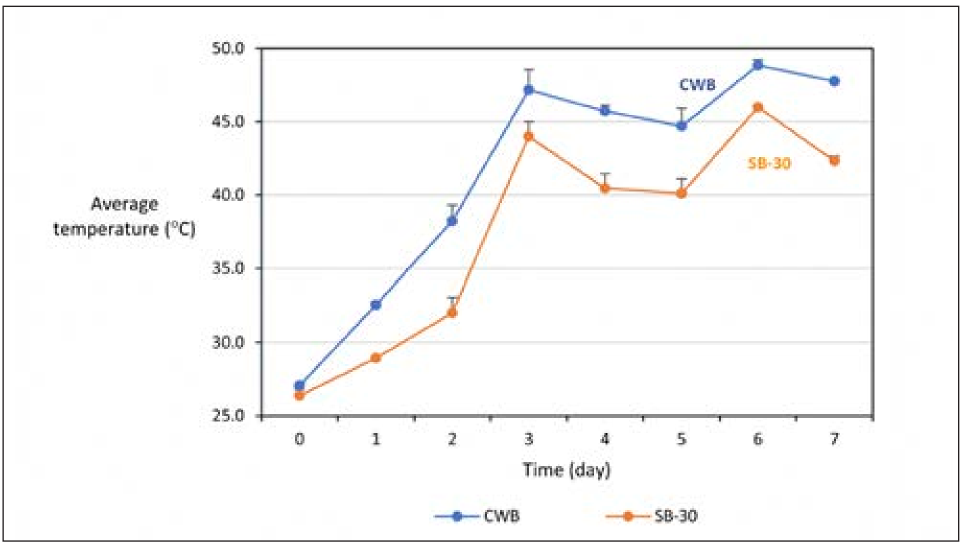

Figure 3: Comparison of average temperature trends as time progressed during cacao fermentation in Commercial Wooden Box (CWB) and SB-30 prototype.

Average pH of testa and cotyledon trends were almost identical in case of testa and very similar in the case of cotyledon for the two treatments (Figure 4). The cotyledons acidified mainly after the first turning in the case of SB-30, but showed a consistent acidification trend in CWB.

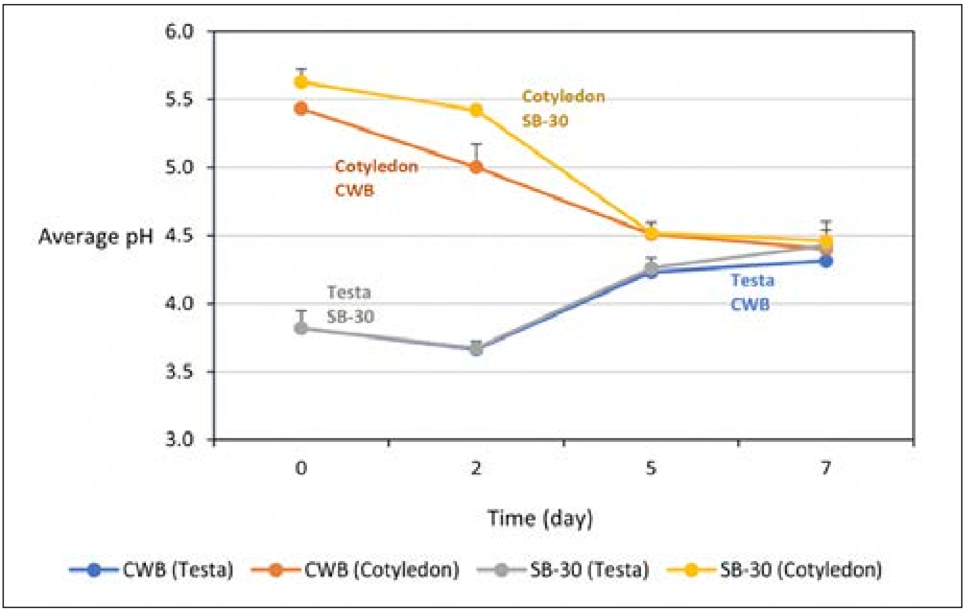

Figure 4: Effect of time on average pH of testae and cotyledons as cacao fermentation progressed in Commercial Wooden Box (CWB) and SB-30 prototype.

External bean colour (L,a,b) revealed that lightness (L) was significantly ($P \leq 0.05$) affected by fermentation time (days) by two orders of magnitude compared to treatment effects (Table 2). Beans fermented in CWB were darker than those fermented using the SB-30 prototype. There were no significant differences in terms of treatment on the red (a) and yellow (b) hues ($P \geq 0.05$). Impacts of time (day) were significant for L, a, and b. Interaction effects were also significant implying an influence of time (day) fermented on colour parameters which was treatment specific.

Table 2: Summary of significant effects for external bean colour measured as L,a,b for beans fermented using CWB compared to SB-30 prototype.

Factor	L		a		b	
	Sig.	F value	Sig.	F value	Sig.	F value
Replicate	11.71	***	0.11	NS	4.62	*
Treatment	9.70	***	2.54	NS	0.62	NS
Day	265.49	***	75.07	***	31.63	***
Treatment × day	1.24	*	4.13	**	0.17	NS

NS - Not significant at ($P>0.05$). *, **, *** refer to significance at $P \leq 0.05$, $P \leq 0.01$, $P \leq 0.001$ respectively.

Sensory analyses carried out from liquors prepared at 2, 5 and 7 days of fermentation revealed that cocoa, acid, fresh fruit and floral attributes were significantly (P≤0.05) affected by the treatments (CWB versus SB-30- there was a significant difference between samples generated using each method), (Table 3). Therefore, these attributes were focused on more closely. Panellists' scores for multiple sensory replicates across fermentation replicates were used to generate averages and standard errors graphically depicted in Figure 5 for further investigation.

Table 3: Summary of significant effects across flavour attributes for sensory samples generated for comparison of CWB versus SB-30 prototype.

Factor	Treatment		Day		Treatment × Day	
	Sig.	F value	Sig.	F value	Sig.	F value
Cocoa	8.87	**	7.16	**	2.44	NS
Acid	24.34	***	25.14	***	9.84	***
Astringent	3.23	NS	5.17	**	1.58	NS
Bitter	1.27	NS	5.52	**	2.02	NS
Fresh fruit	34.00	***	20.89	***	11.91	***
Browned fruit	0.69	NS	6.46	**	3.96	*
Floral	21.29	***	0.06	NS	6.56	**
Nutty	0.35	NS	0.36	NS	1.22	NS

NS - Not significant at (P>0.05). *, **, *** refer to significance at P≤0.05, P≤0.01, P≤0.001 respectively.

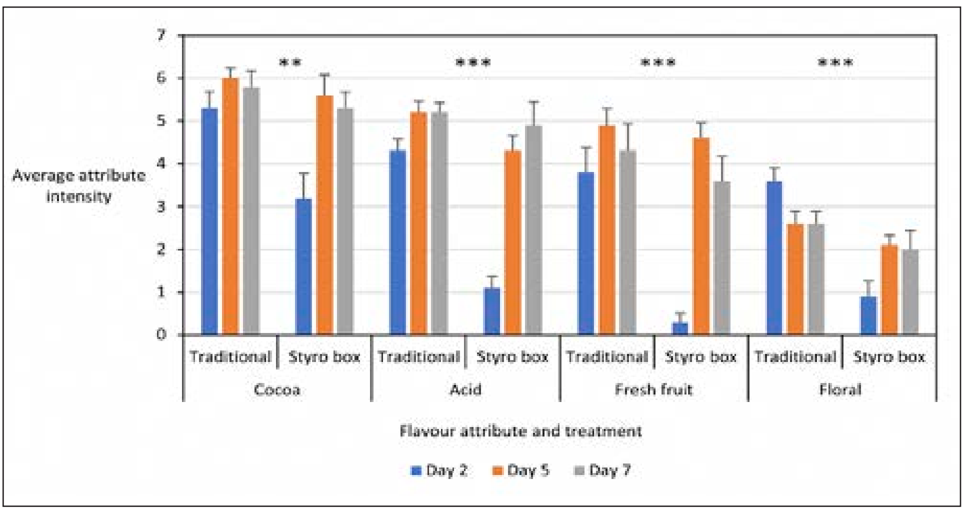

Figure 5: Comparison of average flavour profiles with significance of effect of treatment CWB versus SB-30 prototype.

The cocoa beans fermented for two days were still too bitter and astringent and hence, although the fruity and floral flavours were expressed, the 2-day fermented beans would not have been acceptable for chocolate making. Of the 5 and 7-day fermented samples, the 5-day samples showed significantly higher fruity notes than the 7-day fermented samples in both CWB fermentation and SB-30 optimised protocol. The intensity of the fruity flavour was not significantly different ($P>0.05$) between CWB and SB-30 at 5 days of fermentation. For floral, there was no significant difference between 5- and 7-day fermented samples in both CWB and SB-30. However, the floral note had a small but significant expression in CWB compared to SB-30 prototype. The acid notes were significantly lower ($P\leq 0.05$) in SB-30 at day 5 compared to CWB. Both CWB and SB-30 showed a fermentation optimum of 5 days and showed very similar flavour profiles with small differences in acidity and the intensity of floral note.

Discussion

When SB-5 and SB-30 prototype, fermentations were compared, SB-30 fermentations exhibited a higher temperature overall and temperature peaks and patterns most similar to those observed in successful larger-scale CWB fermentations, that is, two distinct temperature peaks on days subsequent to turning. Turning was done on days three and five of fermentation; and peaks (≥50°C) were observed on days 4 and 6 of fermentation for SB-30. In contrast, The SB-5 fermentations appeared to have decreases in temperature around day 2 of fermentation, clearly indicative of some difficulty maintaining the required conditions and although the temperature peaks were sufficient for bean death, the prevailing trends were lower compared to SB-30. Furthermore, subsequent to the second turning on 5 days a second peak was not observed in SB-5 (as seen in SB-30). Also, there was more variability among zones and replicates in SB-5 fermentations (larger error bars compared to SB-30 averages). The pH of the SB-5 fermentations also increased to 5.0–6.4 by day 7, which is higher than 4.0–5.0 typically observed for that time point in fermentation (Wood and Lass 1985). This can be linked to the increased heat loss and oxidation associated with the small volume and larger surface to volume ratio (indicated by temperature decreases observed) and overall change in the required fermentation dynamics (De Witt 1952). Sensory results show that SB-30 fermentation samples were scored higher for ancillary flavour attributes associated with proper fermentation compared to fermentation samples from SB-5.

Comparison of CWB and SB-30 revealed that the SB-30 produced consistent, acceptable results. The flavours produced were comparable to that of CWB. However, temperatures were consistently lower in SB-30 and pH trends signalled that acetification occurred at a slower rate in SB-30. It is possible that both differences observed can be attributed directly to the effect of a smaller mass size and less pulp containing acids and other chemicals which penetrate the bean and affect the intensity and rate of biochemical processes. As would be expected there was significant variation in day-to-day colour changes (due to varying rates of fermentation between SB-30 and CWB). There was also some significance of replicate effect (L and b values) in both types of fermentation which shows that colour progression was not uniform, as oxidation effects and other factors affecting colouration may have had varying impacts on the same composite bean sample comprising beans withdrawn from different points in the box and exposed to different levels of colour change, however the magnitude of the significance between replicates from the same sample is not large. External colour changes are linked to oxidation and oxidation changes typically start at the top of a box or on the surface of a heap and gradually penetrate the mass of beans, the rate at which this takes place depending on the dimensions of the mass and the turning regime (Wood and Lass, 1985).

Fermentations in both CWB and SB-30 were turned (aerated and homogenised) on days 2 and 5, as this is the industry best practise for traditional CWB fermentation. Therefore, an earlier first turn is carried out and, for comparison purposes, this standardised turning regime was applied across treatments. The fermentation progression was slightly delayed in SB-30 compared to CWB as indicated by the temperature and pH curves. Due to the lag phase, turning at 3 days as opposed to 2 days would likely have benefited SB-30 fermentations (as done in first phase when comparison was made to SB-5). Despite these differences the final quality and flavour attributes were not significantly different between SB-30 and CWB, although the optimum flavours took longer (7 days compared to 5 days in CWB) to develop. It is however noteworthy that the study was able to achieve comparable quality with a 30.0 kg mass (SB-30) compared to a 1000.0 kg mass used in CWB.

Conclusion

When compared to SB-5, the SB-30 prototype fermentations exhibited temperature and sensory trends indicative of more complete fermentation. When managed properly, the SB-30 fermentation protocol gives results comparable to the CWB.

Acknowledgments

We thank staff members of the International Cocoa Genebank Trinidad (ICGT) and staff members of the Cocoa Research Centre, The University of the West Indies, St. Augustine Campus, Trinidad and Tobago, West Indies.

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Journal of Arts, Science and Technology

The Impact of Land Management on Soil Health in Jamaica

A Case Study of the Millbank Farming Region

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Abstract

Land represents an important carbon pool as it provides a range of climate change mitigation and adaptation options based on its management. In addition to carbon sequestration and carbon exchange fluxes, well-managed land supplies a raft of critical ecosystem services. In this case study, we examined numerous abiotic factors as proxies to decipher the impact of land management on long-term soil health at the local spatial scale (tens to hundreds of meters) in rural farming communities of the Upper Rio Grande Valley, Jamaica. The results indicate significant differences in the parameters studied for managed and unmanaged lands. For instance, the soil organic matter content (19.22%) and soil carbon stock (250.13 Mg C ha^{-1}) of unmanaged land are significantly ($p \leq 0.0001$) higher than that of managed land (10.96%; 180.07 Mg C ha^{-1}, respectively). Conversely, soil respiration and carbon loss rates were lower for unmanaged land (40.66 Mg ha^{-1} yr^{-1}; 11.09 Mg CO_2-C ha^{-1} yr^{-1}, respectively) compared with managed land (50.21 Mg ha^{-1} yr^{-1}; 13.69 Mg CO_2-C ha^{-1} yr^{-1}, respectively). Additionally, soil redistribution rates were lower for unmanaged land (0 to -6 t ha^{-1} yr^{-1}) when compared with managed land (-7 to -75 t ha^{-1} yr^{-1}). Overall, these findings demonstrate the potential effects of anthropogenic activities on the contribution of land to deliver ecosystem services sustainably.

Keywords: Case Study, Land Degradation, Land Management, Managed Land, Millbank Farming Region, Soil Health, and Unmanaged Land

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Introduction

Land represents a significant carbon pool as it provides a range of climate change mitigation and adaptation options based on its management (IPCC, 2018). In addition to carbon sequestration and carbon exchange fluxes, well-managed land supplies a raft of critical ecosystem services. These include providing food, feed, timber, and potable water; and preventing or reducing the impacts of natural (weather-related) hazards (IRP, 2019; Vergara et a.l, 2015; UNCCD, 2019; de Moraes Sá et al, 2020a; de Moraes Sá et al, 2020b). However, the accelerated geographic spread of the human use of ice-free land is far greater than any other time in human history with significant implications for nature's contribution to people (UNCCD, 2019; IPCC, 2018). Over the next two to three decades, soil degradation processes such as erosion, depletion of soil carbon and nutrients; unsustainable food production practices (for example, over-fertilization); biodiversity loss; climate change; rapid population growth; and socioeconomic pressures are expected to further imperil the multitude of ecosystem services provided by managed and natural ecosystems (Lal & Follett, 2009; IPCC, 2014). Additionally, land degradation processes are projected to trigger competition for scarce resources leading to migration and the exacerbation of common economic and social vulnerabilities, already evident in climate matters.

Modern agricultural land-use activities (cropland/ pasture) have significantly reshaped the landscape architecture and occupy more than one-third of the global land area (Erb et al., 2018). While social and economic benefits from agriculture have been substantial, agricultural activities are also recognized as a primary driver of local and global environmental change (FAO, 2017). In Jamaica, the updated National Communication indicates that agricultural activities (including land-use changes) account for roughly 40% of the local greenhouse gas inventory (Ministry of Economic Growth and Job Creation Climate Change Division & Convention, 2018). Traditionally, subsistence farming (characterized by highly diverse cropping systems) has been an integral component of rural livelihood and national food systems in the global south (Latham et al., 2017; Wittman & Blesh, 2017). Critically, indigenous and local knowledge has played an integral role in allowing small-scale farmers to continue food production while observing sustainable soil management practices. However, mounting evidence suggests that the future of small-scale farming is increasingly under threat from rural transformation. This transformation has led to a considerable dilution of cultural identity and the perceived value of the cultural ecosystem services (human-environment interactions).

Reconciling the long-standing challenges – of achieving food security, soil security, sustainable development (SDGs; 2, 3, 6, 9, 12, 13, 14, and 15), and long-term temperature goals of the Paris Agreement – calls for an integrated and formidable approach at all scales (IPCC, 2018; Riahi et al, 2017; Friedlingstein et al, 2020). Overcoming these long-standing challenges requires combining land use planning and sustainable land management (including water bodies). Additionally, achieving land degradation neutrality (LDN), gender equality, and access to land would simultaneously reduce waste and pressure on land without compromising the efficacy of food production systems at the local and national levels (UNCCD, 2019).

Considering the current health status of global soils and the continuous pressure of land to deliver crucial ecosystem services, any approach to resolve these issues must, at the very minimum, be research-driven, specifically research focused on soil health (the continued capacity of soil to deliver vital ecosystem services). In this case study, we examine the variation in a host of abiotic factors as proxies to decipher the impact of land management on long-term soil health at the local spatial scale (tens to hundreds of meters) in rural farming communities of the Upper Rio Grande Valley (Millbank farming region), Jamaica. We anticipate that the results of this and related studies will promote the necessary transformation of how land is used sustainably at the local, national, and regional scales to build resilience. Additionally, these data sets will support efforts to achieve the 2030 development agenda and to meet GHG emissions reduction targets under the Paris Agreement.

Materials and Methodology

Site Description and Sample Collection

Jamaica has a tropical climate with a bimodal pattern of rainfall. Rainfalls for the western, central, and eastern sections of the island are roughly 1800 mm yr^{-1}, 1400 mm yr^{-1}, and 2200 mm yr^{-1}, respectively. The overall long-term (30 years) precipitation is roughly 2000 mm yr^{-1}, and the mean annual air temperature is 28°C (source: Meteorological Service of Jamaica, 2000). Based on topography, Jamaica can be divided into two regions: a highland interior and a flat coastal periphery. Approximately, two-thirds of the island is covered by soils underlain by limestone and the remainder with weathered conglomerates, tuffs, shales, and igneous and metamorphic rocks. The spatial distribution of soil orders in Jamaica follows the US Department of Agriculture classification scheme (Nachtergaele, 2001), and the soils may be divided into four broad groups (Garrett et al, 2004): (1) Highland soils of the Blue and Port Royal Mountains, (2) Upland Plateau soils, (3) Alluvial Plain and Valley soils, and (4) Lowland soils. Approximately 15% of the soils are on slopes ≤5%, and nearly 50% have slopes >50% (Fenton, 1982). As a result of the interaction of rainfall, topography, and diverse parent materials, Jamaica exhibits diverse variations in soil types.

Surface soils (0-30 cm) representing different land-use types (broadly catego-

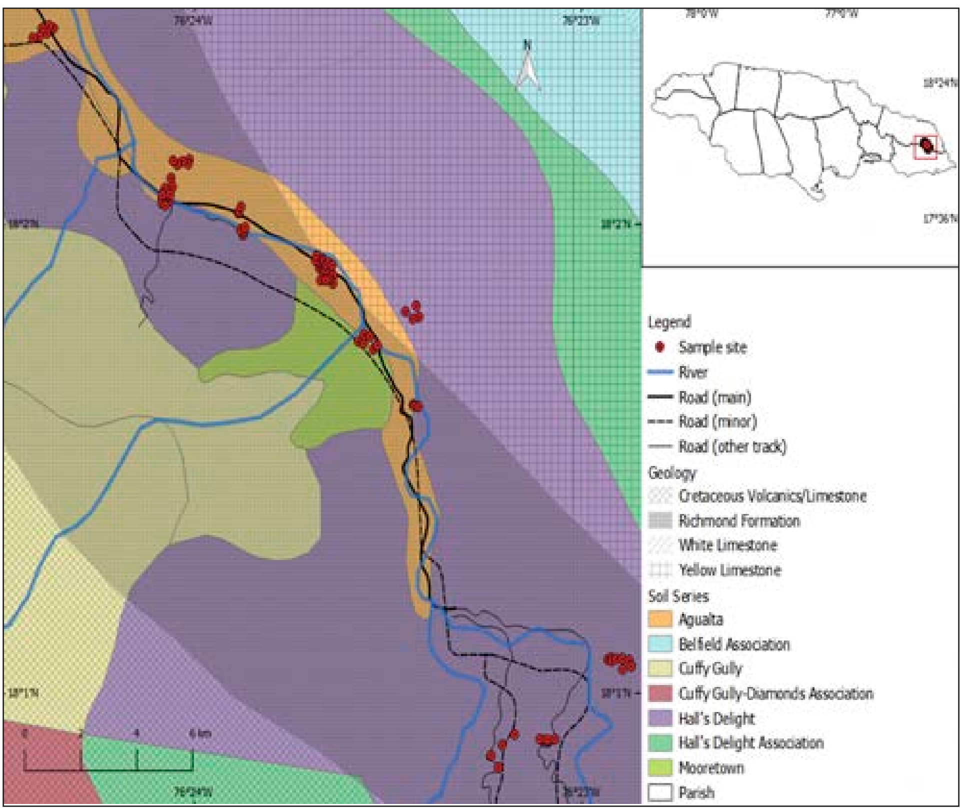

Figure 1: Distribution of soil sample collection sites (stratified according to soil series and geology) in the Millbank farming region, Upper Rio Grande Valley, Jamaica.

rized as managed and unmanaged lands) were collected (January–August 2019) from various sites along a transect (5.9 km, and an area encompassing 340 ha) dominated by *Colocasia esculenta* (dasheen) cultivation (northwest) and a forest reserve (southeast), in the adjoining rural communities of the Upper Rio Grande Valley (18.04°N, 76.40°W). The valley is bordered by Cretaceous rocks (ascending to the Main Ridge of the Blue Mountains) in the southwest, and a Palaeocene limestone escarpment (encompassing the John Crow Mountains) in the northeast (Figure 1). The Highland soil of the valley may be further divided into 1) inceptisols – generally immature, highly porous, acidic, and low in nutrients except in forested areas which allow for the development of an organicrich upper layer; and 2) ultisols and vertisols – less acidic clay-rich soils produced by the weathering of shales in areas with limited draining. The local soil classification is dominated by Hall's Delight series with a textural composition of 19.94% sand, 56.65% silt, and 23.86% clay: and an average bulk density of 0.94 gcm^{-3} for managed land and 0.75 gcm^{-3} for unmanaged land. Although the study area transitioned from a natural ecosystem to one dominated by anthropogenic influences, the climate along the transect is indistinguishable.

Sampling Protocols for Composite Bulk Samples and Erosion Core

The sampling regime involved the collection of composite samples (at each sampling point) using an Edelman hand auger (Spence et al, 2014a). The soil cores were combined in a double-strength polyethylene bag to yield a bulk sample of ~0.5 kg, and the bag was sealed at the collection site. A double cylinder drop-hammer sampler with a 98 cm-3 stainless-steel core was used to sample undisturbed points for soil bulk density analysis. The sampling regime for the erosion cores involved the use of an Eijkelkamp Soil & Water split tube sampler to collect soil cores at a sampling depth of 0–30 cm using the multiple transect approach (Mabit et al, 2014a). All samples were transported to the laboratory under ambient conditions and processed within 24 h of collection.

For the composite samples, each fraction was separately air-dried, disaggregated, and all debris (primarily plant material) removed before being filtered through a stainless-steel sieve with a 2-mm aperture. A small subsample of the <2 mm soil fraction was ground in an agate mortar, and then filtered through a 150 mm stainless steel sieve. The two size fractions (<2 mm and <150 mm) were stored in acid-washed screw-cap polyethylene jars before analysis. Basic information on soil properties was determined using the <2 mm fraction, while elemental analysis was performed using the <150 mm fraction. For the erosion cores, each core was sub-sectioned into 10 cm increments, oven dried at 50°C for 48 h, disaggregated, and all debris removed before being filtered through a 2 mm stainless-steel sieve. A representative sub-sample of each fraction was then analyzed for anthropogenic and naturally occurring fallout radionuclides.

Soil Biochemical Properties

Soil pH, Phosphate, And Nitrate Analyses

Soil pH was determined in a water suspension (soil: solution; 1: 2.5) after shaking at 100 reciprocals min^{-1} for 1 h at ~25°C followed by centrifugation at 2500 rpm for 10 min (Landon, 2014). Phosphate and nitrate concentrations were determined by colorimetry on a Hach colorimeter after extraction in 1 M KCl (soil: solution; 1: 5) with shaking at 175 reciprocals min^{-1} for 1 h at ~25°C followed by centrifugation at 2500 rpm for 10 min and filtration using 0.45 µm PTFE syringe filters (Soil Survey Staff, 2014).

Soil Carbon Stock

Soil bulk density ($g\ cm^{-3}$) was determined by drying the 98 cm^{-3} stainless-steel core containing the undisturbed soil core at 105°C for 48 h in a Vulcan 3550 bench top furnace (Brus et al, 2017; Walter et al, 2016) then applying the equation below:

$$\text{Bulk Density} = \frac{\text{Mass of dried soil}}{\text{Volume of the cylindrical core}}$$

The soil organic carbon (SOC) content was then estimated by dry combustion via loss on ignition 168 (LOI) at 550°C in a Vulcan 3-550 bench top furnace for 4h (Heiri et al, 2001). Loss on ignition ($LOI_{550°C}$) is a physical technique that is frequently used as a surrogate for the organic matter content of soils (SOM) and sediments and has been shown to correlate well with other chemical methods (Heiri et al, 2001). The inorganic carbon (carbonates) content of the soil is not resolved during this dry combustion since the oxidation temperature does not exceed 550°C. The soil carbon stocks ($Mg\ C\ ha^{-1}$) of each land use type were then calculated using the below equation (Poeplau et al, 2017):

$$SOC\ (\text{Mg C}\ ha^{-1}) = \text{SOC}\ (\%) \times \text{BD}\ (\text{g cm}^{-3}) \times \text{depth of soil (cm)}$$

where,

SOC (%) is the fraction of carbon (0.58) in the soil organic matter (SOM) and BD is the bulk density (mass per unit volume of dried soil).

Infrared Spectroscopy

In preparation for infrared spectroscopy, a subset of dried soil samples was repeatedly treated with a 10% hydrofluoric (HF) acid solution. Treatment with 10% HF acid solution effectively concentrates the organic matter and removes silicates and other paramagnetic minerals (Gonçalves et al, 2003). Samples were analyzed immediately after preparation to minimize the suppression of key signals by KBr-adsorbed atmospheric water (Spence & Kelleher, 2012). It is important to note that treatment with HF does not alter the overall soil organic matter composition (Rumpel & Dignac, 2006; Pisani et al, 2015).2015).

Elemental Composition

Sample Irradiation and Analysis

Instrumental neutron activation analysis was performed using the JM-1 SLOWPOKE-2 (Atomic Energy of Canada Limited, ON, Canada) nuclear reactor at the International Centre for Environmental and Nuclear Sciences (ICENS) using the relative method (Kennedy & St-Pierre, 1999). For the determination of short-lived radionuclides, an approximate mass of 0.20 g of solid sample was accurately weighed out in acid-washed polyethylene vials (Polyvials EP 338®) and then heat-sealed. These vials were subsequently encapsulated in larger polyethylene vials (Polyvials EP 290LG®) which were also heat-sealed. These samples were then irradiated at a thermal neutron flux of Φth = 2.5 x 1011 $ncm^{-2}S^{-1}$ for 120 s. Samples were initially given a decay period of up to 600 s before being counted for an acquisition period of 300 s. Samples were counted again approximately 3600 s after irradiation for 600 s (Grant et al., 1998).

For the determination of intermediate and long-lived radionuclides, an approximate mass of 0.25 g of solid sample was accurately weighed out in acid-washed polyethylene vials (Polyvials EP 338 ®) and heat-sealed. These vials were also encapsulated in larger polyethylene vials (Polyvials EP 290LG ®) and heat-sealed. Samples were irradiated at a thermal neutron flux of Φth = 1 x 1012 $ncm^{-2}S^{-1}$ for 3600 s. Samples were counted after 5 days of decay for 3600 s for the determination of intermediate-lived radionuclides. The samples were counted again approximately 21 days after irradiation for 7200 s for the determination of long-lived radionuclides (Grant et al., 1998). All samples were counted on an Ortec High-Purity germanium (HPGe) coaxial gamma photon detector system with a relative efficiency of 71% and a resolution of 1.9 keV at the 60 Co 1332 keV gamma line and a Peak to Compton ratio of 78:1 at the 60Co 1.33 MeV gamma line.

Spectra were acquired using Ortec's Gammavision 6.01 software, and calculations and data were processed using in-house software based on Ortec's Omnigram program.

Gamma-Ray Spectroscopy

The gamma-emitting ^{210}Pb, ^{137}Cs, ^{7}Be, and ^{226}Ra nuclides were measured by counting approximately 10 g of dried pulverized sample in a 10 mL calibrated counting vial for 24 h. The activities of these nuclides were measured by using a high purity germanium well detector coupled to a Canberra DSA multi-channel analyzer (Baskaran et al, 2015). The gamma detector was calibrated with sediment standards [IAEA-300 for ^{137}Cs (661 keV) and RGU-1 for ^{210}Pb and ^{226}Ra (via ^{214}Pb and ^{214}Bi at 352 keV and 609 keV respectively)] obtained from the International Atomic energy Agency (IAEA). The typical resolution (FWHM) of the detectors was about ~1.3 keV at 46 keV and ~2.2 keV at 1.33 Mev (Jweda & Baskaran, 2011)

Determination of Soil Redistribution Rates

The fallout radionuclides' inventories were converted to soil redistribution rates using a suite of models developed by Walling et al, 2011. For ^{137}Cs, we employed the mass balance model 1. On the other hand, for ^{210}Pb and ^{7}Be we employed a modified version of the mass balance model 2, which typically improves on the estimates of the previous model (Mabit et al, 2014b; Benmansour et al, 2014). Additionally, for the unmanaged lands, we employed the ^{137}Cs profile distribution model and the ^{210}Pb and ^{7}Be diffusion and migration model, which account for the gradual diffusion of the radionuclides down the soil layers (Walling et al., 2011).

Soil Flux Measurement

To determine the soil CO2 efflux (μmol CO_2 m^{-2} s^{-1}) variability for managed and unmanaged land, we employed a closed-chamber survey (short-term) system (LI-COR 8100A, LICOR, Lincoln, Nebraska, USA) to perform rapid measurements at several sites (Marwanto & Agus, 2014). All soil flux rates were computed using the data manipulation software, SoilFluxPRO (Licor).

Statistical Analysis

Statistical manipulation of the data was carried out using R Studio and Microsoft Excel 2010 with the XLSTAT 2008 add-on. Due to the non-normal distribution pattern of the data, nonparametric statistical methods were employed. Median values were used as a measure of central tendency, and Spearman's correlations were used as a measure of statistical dependence between variables. The Mann-Whitney U test was used to determine whether observed differences in the median values were statistically significant. All tests for statistical significance were performed at the 95% confidence level.

Results and Discussion

General Geochemical Characteristics

The summary results of selected abiotic factors which are known to, at the local spatial scales, drive multiple functions of soils in the delivery of crucial ecosystem services are presented in Table 1. In general, there are notable (significant) differences between the (bio)geochemical aspects of the samples collected from what we broadly define as managed and unmanaged land.

More specifically, results indicate that soil pH varied widely between managed (4.02 to 7.72; median 5.41) and unmanaged land (4.11 to 6.53; median 5.08). All soil samples are predominantly acidic (92% of samples ≤ pH 6; Figure 2a), which is not surprising despite the primarily calcareous nature of the soil antecedent. It has been shown that a ubiquitous feature of highly weathered local soils is a pronounced depletion in base cations (Ca^{2+}, Mg^{2+}, Na^{+}, and K^{+}) due to leaching, and simultaneous enrichment of Si, Al, and Fe ions in near-surface horizons. (Lalor, 1995; Spence et al, 2014b). Also, this is generally accompanied by weak buffering, exacerbated by anthropogenic activities such as fertilizer application to boost crop productivity. Further, the area studied is best classified as being at an immature stage of ecosystem development and is therefore characterized by shallow OM development and acidic pH values (often averaging pH 4.0) (Spence et al, 2014a; Wardle et al., 2014).

Table 1: Summary of selected physiochemical properties of the soil samples studied

Soil Properties	Median values	
	Unmanaged Land	**Managed Land**
Phosphate (mg L^{-1})	0.15	0.28[a]
Nitrate (mg L^{-1})	2.88	3.98[b]
pH units	4.70	5.11[c]
Organic matter content (%)	18.76	10.96
Soil organic carbon (%)	10.88	6.36
Soil carbon stock (Mg C ha^{-1})	244.14	180.07
Soil CO_2 flux (Mg ha^{-1} yr^{-1})	40.66	50.21
Carbon loss (Mg CO_2-C ha^{-1} yr^{-1})	11.09	13.69

All values are significantly different ($p < 0.0001$) unless otherwise stated
[a] Values were significantly different ($p = 0.047$)
[b] Values were not significantly different ($p = 0.202$)
[c] Values were not significantly different ($p = 0.15$)

Similarly, the concentrations of phosphates (0.15 mg/L to 0.28 mg/L) and nitrates (2.88 mg/L to 3.98 mg/L) varied widely and were significantly different between managed and unmanaged land ($p \leq 0.03$) (Figure 2b and c). The higher concentrations were observed for managed land and may represent a direct indicator of an anthropogenic influence such as fertilizer application commonly associated with agricultural activities. On the other hand, the relatively lower concentrations of phosphates and nitrates observed for unmanaged land may be a function of lower input into these soils from microbial-mediated nitrogen fixation of litter (Crews et al, 2000) and the weathering of phosphatic rocks, respectively.

The soil organic carbon (SOC) – which constitutes roughly 58% of all soil organic matter (SOM) – is another powerful index of soil health. Due to its roles in nutrient turnover and stability, moisture retention, soil structure development, and the regulation of GHG emissions (Don et al, 2013), it is critical in sustaining plant, microbial and animal life (Kögel-Knabner et al, 2005). Here we provide quantitative estimates of SOM/SOC under different land-use systems, topography, and soil types. This (and other) data may then be used to inform site-specific sustainable land management practices, including restoration interventions where applicable. The OM (% LOI) values, SOC contents, and carbon stock of managed land ranged from 6.03% to 16.27% (median 10.96%); 3.50% to 9.44% (median 6.36%); and 99.07 Mg C ha-^{1} to 267.31 Mg C ha^{-1} (median 180.07 Mg C ha^{-1}), respectively (Figure 3).

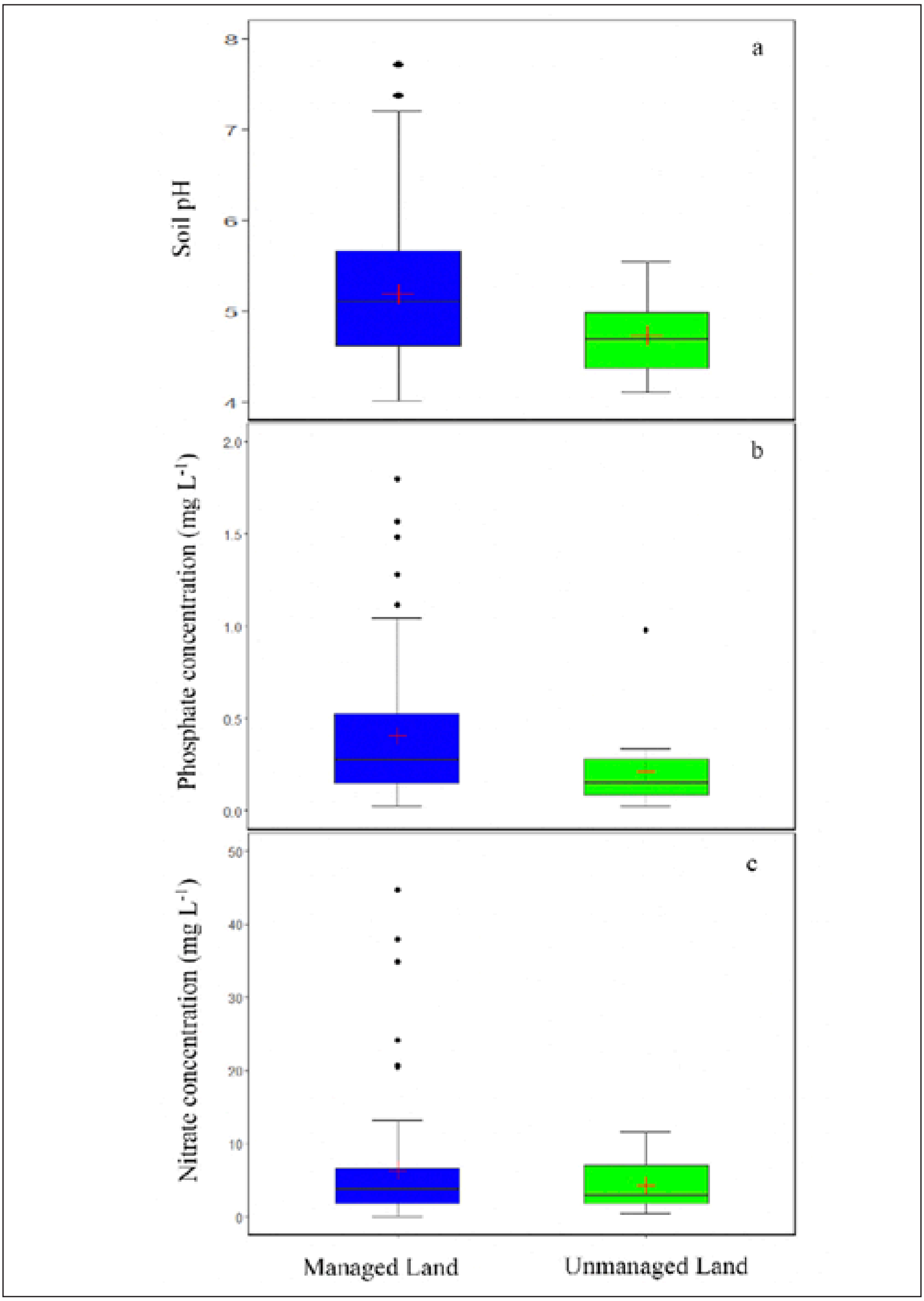

Figure 2: General geochemical characteristics a) pH; *b*) extractable phosphate *(mg L^{-1})*; and *c)* extractable nitrate *(mg L^{-1})* associated with soil samples collected from the Millbank farming region. The 'box and whisker' plot illustrates the first and third quartiles (boxes), median (horizontal lines), mean (red cross), and outliers (solid circle). The 'whiskers' show the range of values that fall within the inner fences (data points that are up to 1.5 times the interquartile range).

Conversely, the OM values, SOC contents, and carbon stock of unmanaged land ranged from 12.64% to 29.00% (median 19.22%); 7.33% to 16.82% (median 11.15%); and 164.50 Mg C ha^{-1} to 377.41 Mg C ha^{-1} (median 250.13 Mg C ha^{-1}), respectively. The values for the unmanaged lands are significantly different ($p \leq 0.0001$) from that of managed land (Figure 3) and are consistent with the expectation that forest soils generally contain higher OM and OC values compared with cultivated soils (Federici et al., 2008). However, these findings indicate that both the managed and unmanaged soils are of moderate to high fertility.

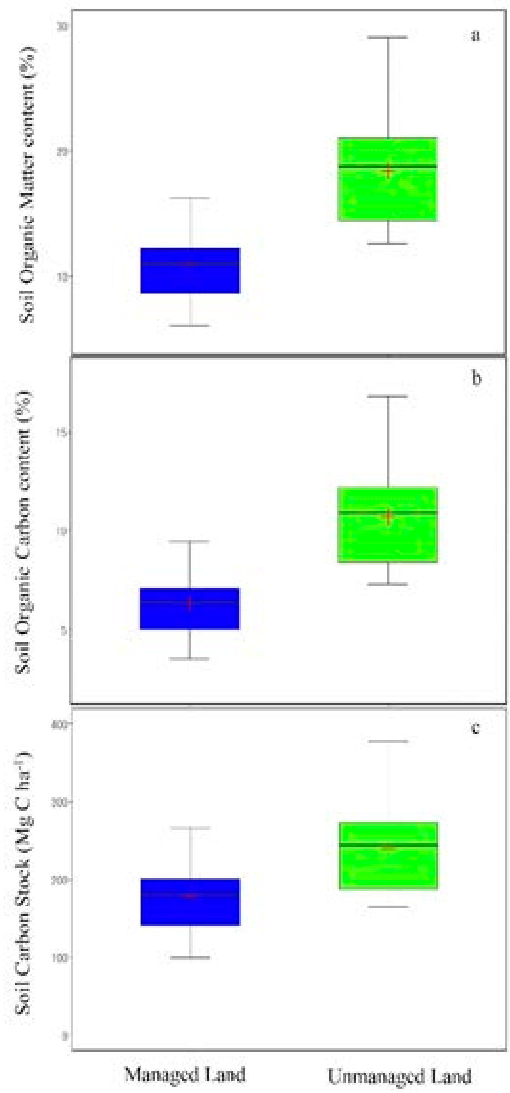

Figure 3: The distribution of: a) SOM content; b) SOC; and c) soil carbon stocks across different land-use types.

In addition to quantifying the soil carbon stocks under different land-use, we also investigated the quality (molecular structure) of the carbon present in these stocks to make reasonable inferences about the local soil health, as well as the carbon sequestration and climate change mitigation potential of these land-use types. To achieve this, we employed Infrared spectroscopy. Before any further discussion is taken, readers are reminded that the results presented in this section are from HF-treated and untreated soils. In the case of the untreated soils, we can study possible organo-mineral interactions; while the HF-treated samples emphasized the organic structures present in the samples. The stacked infrared spectra (HF-treated and untreated) of the managed and unmanaged land (Figure 4) represent the primary biomolecular characteristics of soil from these land-use types. The spectra are characterized by two primary regions (3900 cm^{-1}–2700 cm^{-1} and 1900 cm^{-1} –400 cm^{-1}), representing a range of functionalities found in a diverse group of organic compounds. Further, the sub-region that extends from 1100 cm^{-1} to 400 cm^{-1} is punctuated by several inorganic components such

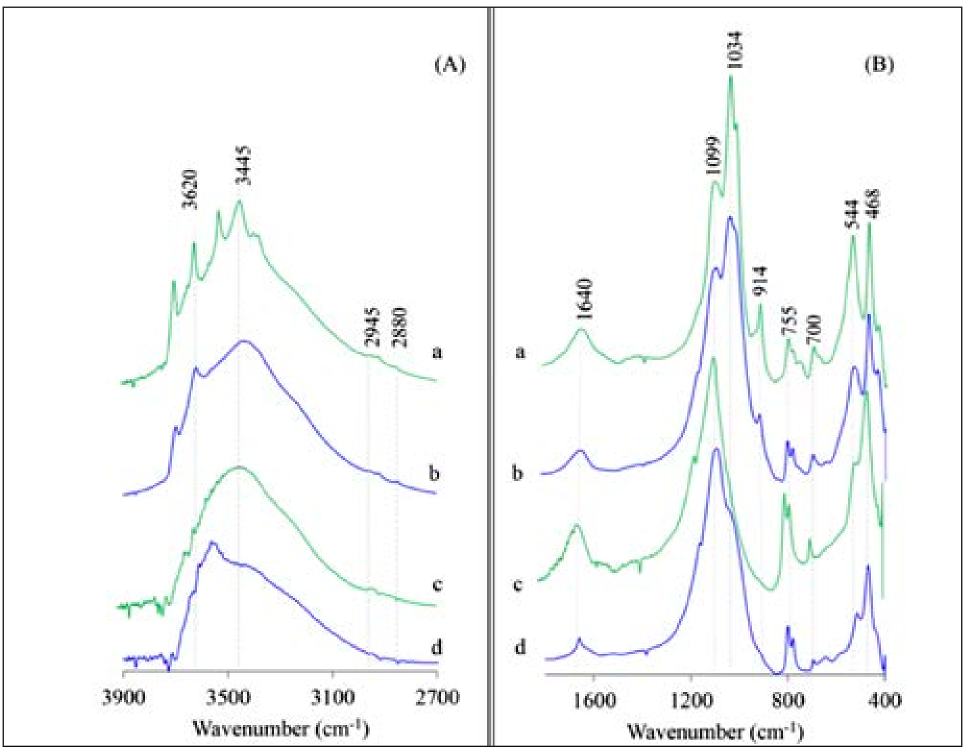

Figure 4: Stacked infrared spectra of a) untreated soils from unmanaged land; b) untreated soils form managed land; c) HF-treated soil from unmanaged land; and d) HF-treated soils from managed land in the OH stretching (A) and 1800 cm^{-1}–400 cm-1 regions (B).

as quartz, clay minerals, and pedogenic oxides. The general peak assignments provided here apply to all spectra and the characteristic functionalities of major biochemical and inorganic components are also summarized in Table 2.

Table 2: Positions and assignments of adsorption bands *(cm^{-1})* observed in the infrared spectra of composite soil samples.

Observed Wavenumber (cm^{-1})	**Assignments**
3620	ν(OH) of hydroxyl groups ν(N—H) of amide A in proteins
3445	ν(N—H) of amide B ν_{as}(O—H) of H-bonded phenolic groups
2945	ν_{as}(C—H) of CH_2 in aliphatic structures
2880	ν(C—H) of CH_2 in aliphatic structures
1640	ν(C=C) in aromatics, *ν*(C=O) in amide I
1099-1095	ν(C—C), ν(C—O), ν(C—O—C) predominantly from ring vibration of carbohydrates ν(P—O) of nucleic acid
1034	ν(Si–O) of quartz (sand)
990-500	δ(=C—H) or aromatic
914	δ(OH) of structural OH in clay minerals
755-700	ν(OH) surface hydroxyl groups in clay minerals
800	ν(Si—O)
544	δ(Si—O—Al)
468	ν(Si—O—Si)

ν- Symmetric stretching
ν_{as}- Asymmetric stretching
δ – Bending

The broad, but reasonably well-defined band (3610 cm^{-1} - 3300 cm^{-1}) with a peak maximum positioned near 3445 cm^{-1} is indicative of ν(OH) in phenolic compounds (e.g., phenolic amino acids), and/or carboxylic OH groups as well as ν(N-H) of amides. It is also likely that signals from OH groups are associated with the carboxylic groups of aliphatic lipids and proteins, as well as the surface OH groups and carbohydrates (Spence & Kelleher, 2012; Spence et al., 2014b). Interstitial water molecules that typically surround calcium, magnesium, potassium, and/or sodium ions may also resonate in this region. The presence of these dibasic cations enhances the buffering capacity of the soil (Lalor, 1995; Spence et al, 2014b; Spence, 2019). Additionally, two well-resolved, albeit narrow bands, are observed near 3620 cm^{-1} and 914 cm^{-1} in the untreated samples and support the presence of structural OH groups in clay minerals (Figures 4a and b). This is significant as clay-organo interactions play a crucial role in the long-term stability of otherwise labile OM (Spence & Kelleher, 2012). Note, however, that after HF treatment these bands have diminished, suggesting that these mineral components are susceptible to acidic environments. Soil acidification (natural and anthropogenic) is also a significant contributor to land degradation and usually results from a substantial increase in available Al^{3+} ions, which inhibits the growth of plant roots (Prescott et al., 2021).

Two weak shoulders centered near 2945 cm^{-1} and 2880 cm^{-1} are assigned to asymmetric stretching vibrations from CH_2 and symmetric stretching vibrations from CH_2 methylene groups, respectively. Alkyl groups have been shown to preferentially interact with clay minerals (Spence & Kelleher, 2012). Moreover, the ratio of the bands observed near 2945 cm^{-1} and 2880 cm^{-1} can be used as a proxy to soil moisture retention capabilities, SOM molecular complexity, and SOM stability (Ma et al., 2021). Overall, the relative intensity of major biochemical groups in the 3900 cm^{-1} to 2700 cm^{-1} region of the spectra appears comparable. Phenolic groups and aliphatic components found in SOM also help to stabilize soil humic substances; interact with heavy metals to mobilize cations and regulate toxicity levels; and influence nutrient dynamics, pH, ion-uptake, and soil aggregation (Trevisan et al., 2010).

A moderate band observed near 1640 cm^{-1} indicates coordination between carboxylic groups in biomolecules and minerals and/or ions in the soil (Dick et al., 2003; Celli et al., 1997; Kaiser et al., 1997). The absorption peaks positioned at 1099 cm^{-1}–1095 cm^{-1} also confirm other labile organic structures such as C–O–O and C–O of carbohydrates. Also, signals assigned to sugars, as well as signals resulting from other motions such as P=O symmetric stretching vibration of nucleic acids, are found here (Spence et al., 2013). Given the relative intensity of these peaks in the samples and the labile nature of carbohydrates, this would suggest that these components are physically protected from management-induced degradation and are most likely due to their interactions with mineral oxides/hydroxides (Abdelrahman et al., 2016). Alternatively, the relative intensity of a peak could be a function of the degradation of a more labile component. However, we favor the former explanation as carbohydrates are among the most labile water-soluble biomolecules. On these bases, the results would suggest that soil from both land-use types exhibits good health.

A well-defined band representing crystalline Si—O is positioned at 1034 cm^{-1}. After acid treatment, this band is attenuated while the relative intensities of Si—O (800 cm^{-1}) and Si–O–Si (468 cm^{-1}) vibrations have increased (Figures 4c and d), suggesting that the silicates which remain after HF treatment are now in an amorphous partly protonated silica phase (Spence et al, 2014b). Additional bands near 700 cm^{-1} and 755 cm^{-1} are assigned to surface OH groups in clay minerals. Additionally, the absorption peak due to Si–O–Al vibrations (544 cm^{-1}) has also been diminished after HF treatment (Spence & Kelleher, 2012). The acid lability of Al in mineral structures has previously been demonstrated (Spence et al., 2014b). Overlapping band downfield of 1000 cm^{-1} may also be representative of aromatic components found in biomolecules (Spence et al., 2013).

Elemental and Radiometric Analyses

Given the diverse parent material and soil forming processes typical of Jamaican soil (even within the same ecological zone), we employed Fe/Na concentration ratio as a quantitative measure of soil development within the study area (Garrett & Lalor, 2005; Garrett et al., 2008) This was done as we believe that both pedological and geochemical soil development may influence function. The statistical distribution of the Fe/Na concentration ratio of the soil samples extends over two orders of magnitude and appears discontinuous with one discernible flexure (Fe/Na = 40) indicating two possible sub-groups: Fe/Na <40 (with 89% comprising of managed sites and 11% on unmanaged sites) and Fe/Na = 40–320 (with 40% comprising managed sites and 60% of unmanaged sites) (Figure 5). Each subgroup implies a difference in the maturity of soil development and ranges from immature (Fe/Na <40) to moderately mature (Fe/Na 40–320). Typically, as soils age and mature in humid tropical environments base cations (Ca^{2+}, Mg^{2+}, Na^{+}, and K^{+}) are depleted due to leaching, and Si, Al, and Fe are simultaneously enriched in near-surface horizons. This phenomenon is especially prevalent in Terra Rossa

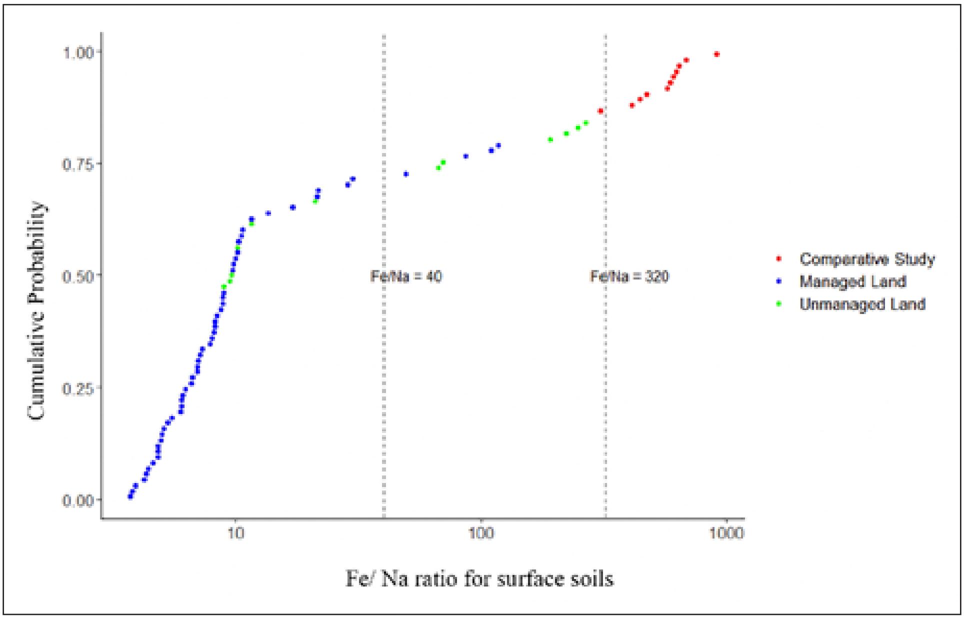

Figure 5: Cumulative probability plot of Fe/Na concentration ratio measured using the *<150-μm* fraction of surface soils collected from managed land and unmanaged land. The Fe/Na concentration ratio for a set of soil samples collected from a bauxite mining plant (unpublished data) is also presented for comparison.

soils (Churchman, 2000), which account for approximately two-thirds of the total surface area of Jamaica (Garrett & Lalor, 2005; Garrett et al., 2008). From our study sites, approximately 85% of all soils collected were classified as Terra-Rosa (Fe/Na <40), and the remaining 15% are classified as non-TerraRosa (Fe/Na 40–320). The physicochemical properties of Jamaica soils have been extensively studied due to their extraordinary geogenic concentration of cadmium (Lalor, 1995; Garret et al., 2010; Spence et al., 2013). Therefore, in addition to our case study, we included the Fe/Na concentration ratio from another study (unpublished data) of more mature soils (bauxitic soils) Fe/Na >320. The comparison highlights that none of the soils from this study had reached the most advanced stages of development.

To further constrain the possible factors that may influence soil health and function, we combine data from a suite of rare earth elements (REE). REE, based on ionic potential, are considered to be proxies of pedogenic environmental processes (Rollinson, 1993). Figure 6 represents the chondrite-normalized REE concentrations of samples collected from the two land-use types. It is apparent that although the REE concentrations vary widely, the patterns between managed and unmanaged land are indistinguishable and are confirmed by REE fraction-

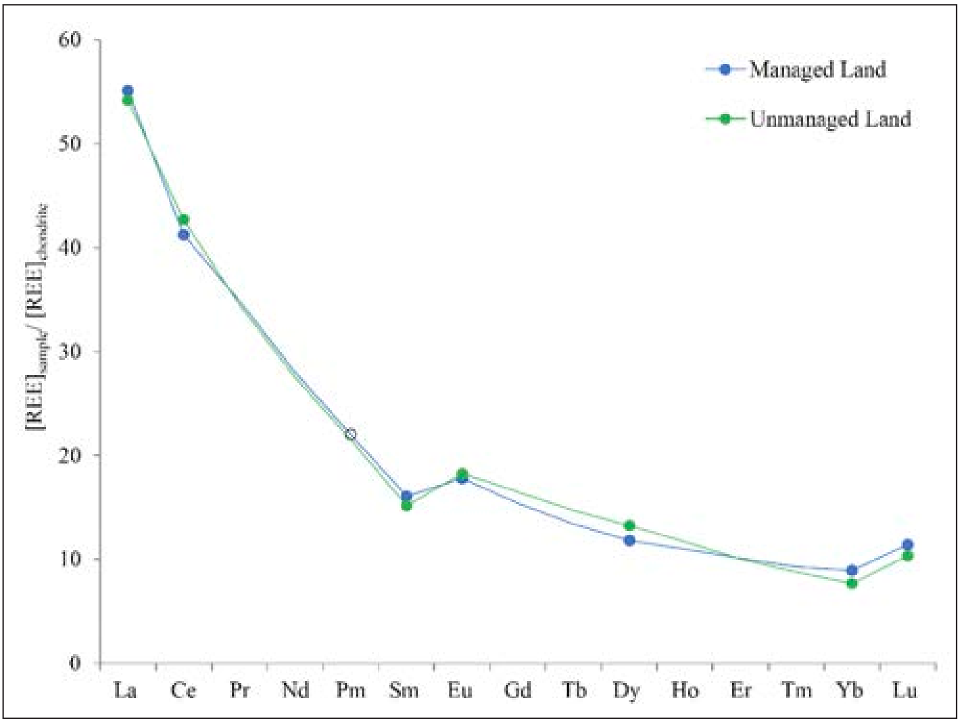

Figure 6: Chondrite normalized REE concentrations of soils collected from the study area in the Millbank farming region. Concentrations vary widely and samples exhibit a positive Eu/Eu* and are enriched in light rare earth element (LREE) relative to heavy rare earth element (HREE).

ation plots (Figure 7). This suggests that any degradation observed is likely to be anthropogenic. Samples from both land-use types exhibit a positive europium anomaly (Eu/Eu*) and are enriched in light REE (LREE) relative to heavy REE (HREE). We note that a positive Eu/Eu* may be indicative of marine influence and may be explained in part by the proximity of the Blue Mountains (15 km) to the ocean (Garret et al., 2005).

Erosion is a primary land degradation process that threatens all aspects of land-based ecosystem services (Tsymbarovich et al., 2020). The severity of the impact of soil erosion creates the need for site-specific estimation of soil redistribution rates to mitigate against land degradation (caused by land use and climate change). Here we report on the use of gamma-ray emitting fallout radionuclides (FRNs; ^{137}Cs and ^{210}Pb) to determine sediment transport rates (Mabit et al., 2014a).

The erosion rates are estimated as -7 t ha^{-1} yr^{-1} to -75 t ha^{-1} yr^{-1} on managed land and 0 t ha^{-1} yr^{-1} to -6 t ha^{-1} yr^{-1} on unmanaged lands (Table 3). The variation in estimates is based on the radionuclides used and represents different temporal

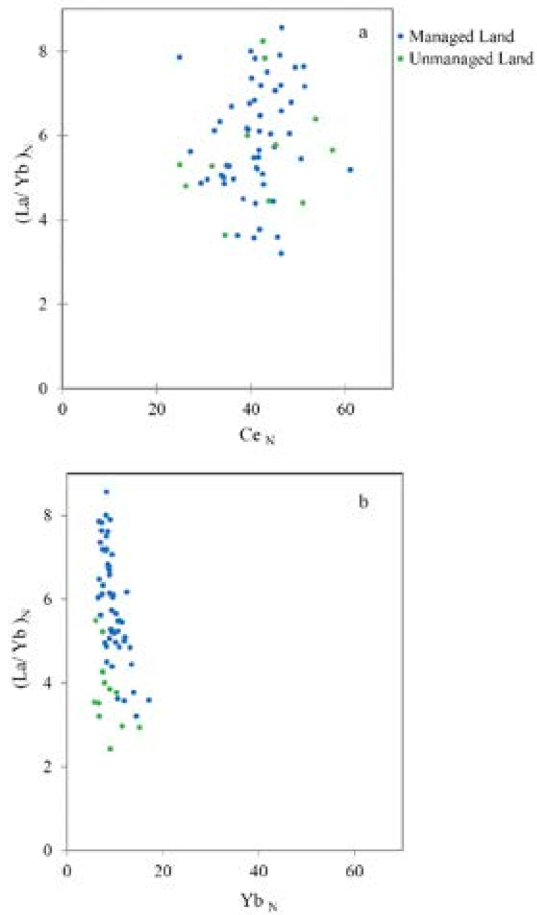

Figure 7: The relationship between a) LREE and b) HREE and LREE/HREE fractionation ratio $(La/Yb)_N$ as a function of land management.

sensitives for each species (Porto et al, 2014; Benmansour et al, 2014). Additionally, the radionuclide distribution throughout the soil profile also provides insights into the dominant land management practices. For instance, we observed peak concentrations at the surface, followed by an exponential decline with sampling depth for the unmanaged lands (Figure 8). On the other hand, we see relatively uniform ^{137}Cs inventory throughout the soil profile for managed lands. The uniform radionuclide inventory suggests soil mixing, most likely due to medium to high-intensity tillage, while the exponential decline in inventory suggests a natural and gradual downward migration of the radionuclide (free of human interferences).

Table 3: Estimates of site-specific soil redistribution rates obtained from fallout and naturally occurring radionuclides within the Millbank farming region.

Land-use type	Soil Erosion Rate (t ha^{-1} yr^{-1})	
	^{137}Cs	^{210}Pb
Unmanaged Land		
Reference Point	0.00	0.00
Site 1	- 6.1	- 1.2
Managed Land		
Site 2	-36.4	-7.3
Site 3	118.1	-1.8
Site 4	-70.1	n/a
Site 5	-75.3	n/a

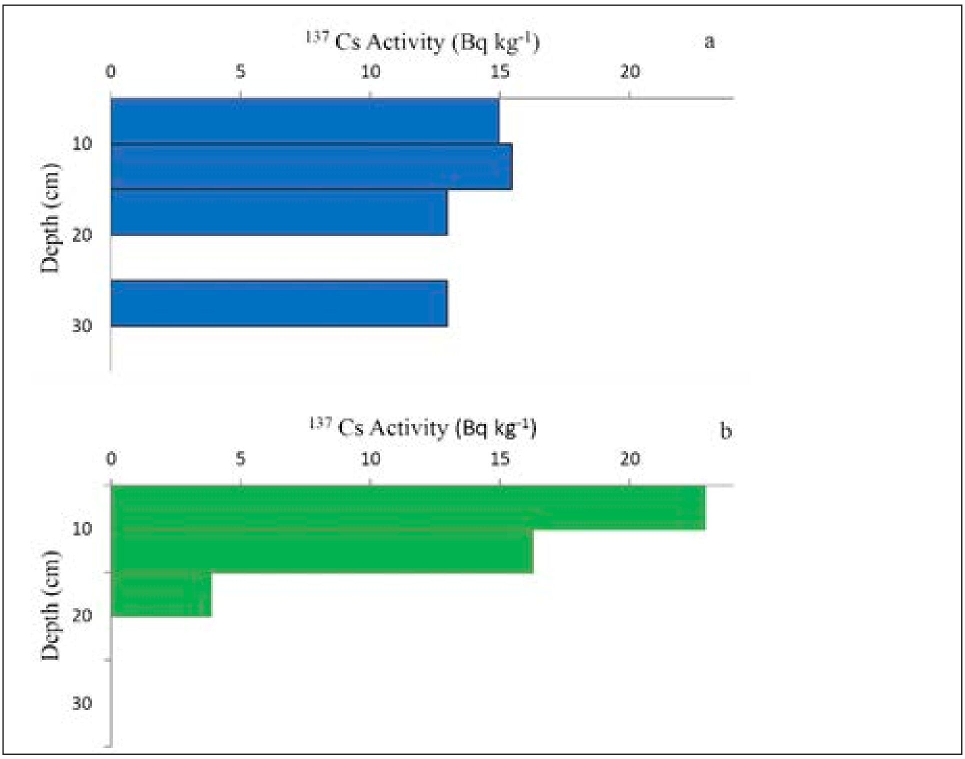

Figure 8: *Vertical distributions of ^{137}Cs (Bq m^{-2}) in soil cores collected from a) managed land; and b) unmanaged land in the study area.*

In most cases, the surface layer contained the highest amount of ^{137}Cs and ^{210}Pbex, except on the slopes of the recent gullies. These slopes contain little to no detectable levels of the radionuclides in the upper 10 cm of the soil suggesting severe erosion or landslides. Concerning climate, we also observed lower ($p = 0.168$) ^{137}Cs activities for managed lands (6.9 Bq kg^{-1}) compared to unmanaged land (16.3 Bq kg^{-1}). It is known that the amounts of fallout generally increase with increasing rainfall (Zhang et al., 1999). Therefore, the higher input observed could suggest subtle microclimatic differences (i.e., higher annual rainfall) in the unmanaged land (e.g., forest reserve) when compared to the managed lands (Chen et al., 1999).

Soil Flux and Carbon Loss

Land-use and land-use changes associated with agricultural activities are recognized as primary contributors to global GHG emissions (IPCC, 2014). Furthermore, it is estimated that approximately 75 Gt C yr^{-1} are lost through soil respiration (Schlesinger & Andrews, 2000). Cumulatively, this has important implications for atmospheric CO_2 concentrations and global climate. We, therefore, monitored soil respiration (CO2 fluxes) on unmanaged and managed sites to evaluate the effects of land management practices on soil, carbon turnover, and soil health. For ease of comparison, all sites were selected to fall within a 2 km radius with comparable geology and topography. The managed sites that met these requirements were under a minimum tillage regime. The average carbon losses were then determined from the flux values and are displayed in Figure 9.

Soil respiration for unmanaged lands (40.66 Mg ha^{-1} yr^{-1}) (mean value with error) was remarkably lower ($p = 0.0002$) than that observed for managed lands (50.21 Mg ha^{-1} yr^{-1}). This may be attributed to the limited size of the labile carbon pool and the reduced aeration associated with the absence of tillage on the unmanaged land. We also considered the high-resolution magic angle spinning (HR-MAS) and diffusion editing (DE) NMR spectra of SOM from forest soils obtained from a previous study. The spectra revealed that the SOM from forest soils are dominated by recalcitrant molecular structures (e.g., cutin and suberin) existing predominantly as large macromolecular compounds (Pisani et al., 2015). In DE NMR, small molecules are essentially gated from the final spectrum but signals from macromolecules that display little translational diffusion are not gated and appear in the spectrum (Simpson et al., 2007).

It is also important to note that soil respiration is an inherently complex process

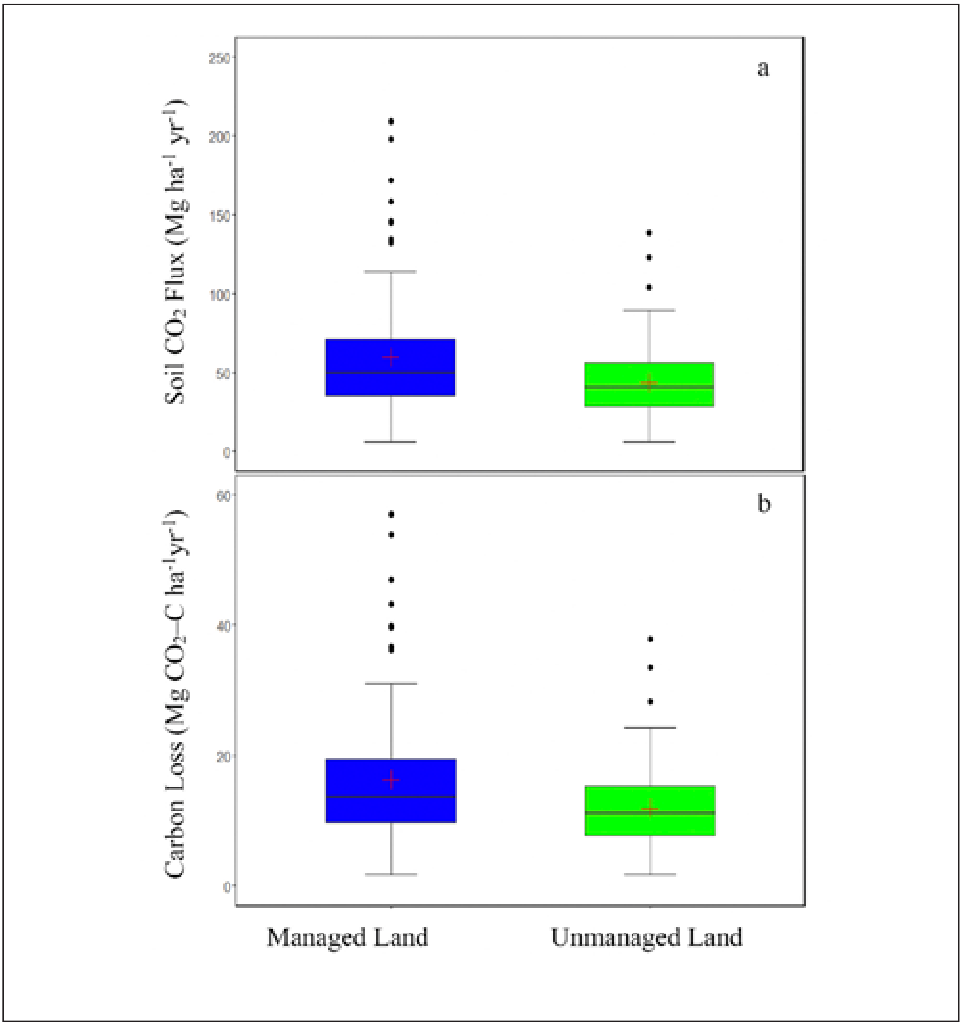

Figure 9: Soil respiration rate *(Mg CO_2 ha^{-1} yr^{-1})* (a) and carbon loss *(Mg CO_2-C ha^{-1} yr^{-1})* (b) as a function of land management.

and exists within a plant-soil continuum (Högberg & Read, 2006). Therefore, the contribution of root-derived (autotrophic) and microbial-derived (heterotrophic) respiration may vary significantly, despite our best efforts to avoid root shearing during collar placement. The variation in measurements from different land-use types may also reflect different levels of impact by environmental factors such as temperature, humidity, and/or soil moisture. For instance, it has been shown that taking daytime measurements coupled with different extents of root shearing may underestimate the autotrophic component of the soil flux (Heinemeyer et al., 2011).

Conclusion

Agricultural land use and food production are major transgressors of multiple planetary systems. For example, land-use and land-use change are proposed to alter SOC biogeochemistry and nutrient status, leading to shifts in soil-atmospheric carbon fluxes and agricultural productivity. Notwithstanding, the land remains a viable option for climate change mitigation (with adaptation co-benefits) through emissions reduction and carbon dioxide removal (CDR). Therefore, a comprehensive transformation of agriculture and food systems, starting at the community level, is required to achieve the double helix of the 2030 developmental goals and the long-term temperature goals of the Paris Agreement. In this case study, several significant differences are noted in the general geochemical characteristics (e.g., SOM, SOC), elemental contents, radiometric profiles, soil respiration rates, and carbon losses of soils under different land management practices in the Upper Rio Grande Valley. These findings have important implications for sustainable agriculture, food security, and several other ecosystem services, particularly biodiversity and climate change mitigation and adaptation.

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Journal of Arts, Science and Technology

Temperature and Chemical Induced Conformational Changes in Purified PEPC

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Abstract

As a key enzyme in C4 metabolism, the enzyme phosphoenolpyruvate carboxylase (PEPC) is of interest to numerous plant scientists working to improve photosynthesis around the world. Temperature caused marked and reversible changes in the properties of the PEPC protein purified from A. hypochondriacus leaves. There was no difference in the extent of phosphorylation of PEPC in A. hypochondriacus leaves when exposed to different temperatures, in contrast to the marked increase in phosphorylation of enzyme when the leaves were illuminated. The presence of PEG-6000 was effective in protecting PEPC against cold inactivation, indicating the importance of the conformation of PEPC. Non-denaturing PAGE of purified PEPC revealed the existence of three different forms of proportionally increasing molecular weight: monomer, dimer and tetramer. Cold temperatures tend to shift the equilibrium of the PEPC protein toward the tetramer. The presence of PEG and/or glycerol resulted in a dominance of tetramer. Using intrinsic/extrinsic fluorescence estimation and CD spectroscopy, the current work reports the influence of temperature on the secondary and tertiary structure of the PEPC of Amaranthus hypochondriacus. The study shows how temperature fluctuations cause conformational changes. These results suggest that structural changes are a factor in how allosteric effec-

tors and inhibitors affect PEPC function. Studies on the integration of CCM in C3 plants target PEPC as it is a crucial component of the carbon concentration mechanism in higher plants. As a result, studies of its structure and function are expanding our understanding of the protein.

Keywords: PEPC, Amaranthus Hypochondriacus, PEG-6000, Urea, Conformational Changes and Phosphorylation.

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Introduction

Many C_4 species are cold sensitive and one of the factors associated with this phenomenon is the modulation by cold temperature of key enzymes of C_4 pathway, such as PPDK or PEPC (HDu et al., 1999). At low temperature, PPDK, a chloroplastic enzyme is inactivated due to dissociation and such inactivation/dissociation is prevented by glycerol, sucrose or sorbitol (Shirahashi et al., 1978). It is possible that solutes such as glycerol or PEG-6000 can protect PEPC against temperature induced changes (Chinthapalli et al., 2003).

The sensitivity of PEPC to malate is influenced by various factors, such as light, temperature, pH and Glc-6-P. When leaves are illuminated, there is a marked decrease in sensitivity of PEPC to malate besides an increase in the enzyme activity. The changes during light activation are all due to the phosphorylation of the enzyme. We have recently demonstrated that there is a marked decrease in the sensitivity to malate of PEPC in leaf discs, as the temperature was raised from 15°C to 50°C. However, there was no difference in the extent of phosphorylation of PEPC at varying temperature in leaves of *A. hypochondriacus* (Chinthapalli et al., 2003, 2014).

Compatible solutes, like PEG-6000, promote the oligomerization of PEPC, increase the enzyme activity and decrease its malate sensitivity (Huber and Sugiyama, 1986). Protection of enzyme activity against high temperature was shown also in presence of glycerol, although PEG was more effective than glycerol (Drilias et al., 1994). Besides PEPC, other cytosolic enzymes known to be activated by PEG are: pyruvate kinase from germinating castor oil seed endosperm and fructose 1,6-phosphatase (FBPase) (Hodgson and Plaxton, 1995).

PEPC undergoes posttranslational modification by reversible phosphorylation

(Vidal and Chollet, 1997) or shift in oligomeric state of the enzyme (Chollet et al., 1996). Compared to the extensive literature on posttranslational modification of enzyme in C_4 PEPC, studies on conformational changes other than phosphorylation and oligomerization of PEPC are limited. There are events where regulatory phosphorylation is not involved for the increase in PEPC activity and subsequent decrease in malate sensitivity of the enzyme (Chinthapalli et al., 2003). At low temperature the activity of the enzyme decreased and malate sensitivity increased in C_4 plants, and at high temperature enzyme activity increased and malate sensitivity decreased. These changes appear to be due to aggregation of PEPC rather than phosphorylation (Wu and Wedding, 1987; Chinthapalli et al., 2003).

Conformational changes can be studied using different approaches like intrinsic or extrinsic fluorescence. Changes in the intrinsic fluorescence as indication of conformational changes have been recorded in enzymes like PEPC in *Rhothermus Obamensis* (Takai et al., 1997), invertase of yeast (Cavaille and Combes, 1995), thermostable D-glyceraldehyde-3-phosphate dehydrogenase (GAPDH) from the *Thermotoga maritime* (Wrba et al., 1990) and in soyabean peroxidase (Kamal and Behere, 2002). Conformational changes can be studied by also studying the extrinsic fluorescence of dyes, which bind to the proteins. For e.g., fluorescent probes such as ANS (1-anilinonaptha 8-suphonic acid), bis-ANS [1,1'-Bis (4-anilino-5-naphthalenesulfonic acid)] or Nile Red (Takai et al., 1995), are widely used to probe the hydrophobic surfaces of proteins.

Detailed molecular analysis of the structure-function relationships in PEPC have been hampered by the lack of information on its three-dimensional structure. A partial X-ray crystallographic analysis has recently been accomplished for PEPC from *E. coli* (Matsumura et al., 1999a) and C_4 plant, *Zea mays* (Matsumura et al., 1999b; Matsumura et al., 2002). Circular dichroism (CD) spectroscopy measures the difference in absorption of left- and right- circularly polarized light as it passes through optically active or chiral samples. Spectra in the far UV-CD wavelength range (about 190 nm to 250 nm) can provide information on the polypeptide conformation of protein (Wallace and Janes, 2001).

The present study is an attempt to characterize the conformational changes, if any, of PEPC from a typical C_4 plant, *A. hypochondriacus* on exposure to varying temperature. The conformational changes are monitored by recording either intrinsic or extrinsic fluorescence with the help of ANS and CD spectroscopy. Studies were further extended to check these temperature induced changes with those of effectors, compatible solutes, and denaturants like urea.

Materials and Methododology

Plant Material

Plants of *Amaranthus hypochondriacus* L. cv AG-67 were raised from seeds. The plants were grown in earthen pots filled with soil supplemented with farm-yard manure (in a ratio of 5:1). They were grown outdoors in the field under a natural photoperiod of approximately 12 h and temperatures of 30–40°C day/25–30°C night. The upper fully expanded leaves were harvested, about 2–3 h after sunrise and further experiments were conducted.

Figure 1: A view of 4- to 6- old plants of Amaranthus hypochondriacus AG-67, grown in the field (outdoors)

Extraction and Assay of PEPC

Thirty leaf discs (each ca. 0.2 cm 2 and a total weight of 125 mg) were extracted in a chilled mortar and pestle with 500 ml extraction medium containing 100 mM TRIS-HCl, pH 7.3, 2mM $MgCl_2$, 2mM KH_2PO_4, 1mM EDTA, 20% (v/v) glycerol, 10mM b-mercaptoethanol, 10mM NaF, 2mM PMSF, 5mM DTT, and 2% (w/v) insolubles PVP. The homogenate was centrifuged at 15,000 g for 5 min. The supernatant was used as a crude extract. In some experiments, 10 mg per mL Chymostatin was used instead of PMSF. However, PMSF has been used routinely as it has been shown to be very effective in preventing proteolysis in the case of *A. hypochondriacus*. The activity of PEPC was assayed by coupling to NAD Malic Dehydrogenase (MDH) and monitoring NADH oxidation at 340 nm in a Shimadzu UV-Vis Spectrophotometer (Parvathi et al., 2000b). The assay was performed at 30°C regardless of precubation (temperature of leaf discs). The assay mixture (1mL) contained 50mM TRIS-HCl, pH 7.3, 5mM $MgCl_2$, 0.2mM NADH, 2U MDH, 2.5mM PEP, 10mM $NaHCO_3$ and leaf extract (equivalent to 1 mg chlorophyll). The sensitivity of PEPC to malate was checked using either 0.5 mM malate (C_4 species) or 2 mM malate (C_3 species). In some of the experiments (as indicated) 1.25% (w/v) PEG-6000 was also present during the test. Chlorophyll was extracted with 80% (v/v) acetone (Arnon, 1949).

Incubation of Leaf Discs at Different Temperatures

Initial experiments were done with crude extracts by taking thirty leaf discs on distilled water in 5 cm diameter petri dishes and left in the dark for 2 h. After pre-darkening, the leaf discs were incubated for 30 min at the required temperature in the range of 15°C. in a thermostatically controlled water bath to 50°C. The temperature range used for all species was 15°C. to 50°C. At the end of 30 minutes at each temperature, the leaf discs were extracted (as described above) and the extract assayed for PEPC activity.

Purification of PEPC Protein

Purification of PEPC was as described in detail by Gayathri et al. (2000). The total soluble protein was estimated by using Fol1in-Phenol reagent (Lowry et al., 1951), with bovine serum albumin as the standard.

Raising Anti-PEPC Antiserum and Western blotting

Anti-PEPC antiserum was raised in 6 month old white rabbits according to the principles of Nimmo et al. (1986), as described in Gayathri et al. (2001). The labeling of PEPC with 32 Pi in vivo was performed according to the method described by Bakrim et al. (1992). The levels of PEPC protein were assessed by using western blots (Betz and Dietz, 1991), after transferring the proteins electrophoretically from the gel onto the polyvinylidene diuoride (PVDF) membranes (Towbin et al., 1979).

Exposure of Purified PEPC to Varying Temperature

The purified PEPC (30 μg ml^{-1}) was incubated at different temperatures (15°C. to 50°C.) for the required time intervals (0 to 60 min) before assay at room temperature. An aliquot (containing 0.1 g protein) of PEPC was used to assay activity. Unless otherwise stated, PEPC was incubated for 45 min at different temperatures. In experiments to check reversibility, the purified PEPC incubated at an optimal temperature of 40 °C. was treated as a control. The PEPC protein was exposed to different temperatures (for 45 min) and then brought back to the optimal temperature of 40°C. for the next 45 min. The protein was immediately assayed for PEPC at room temperature (30°C.).

Intrinsic and Extrinsic Fluorescence of PEPC

The intrinsic fluorescence of the PEPC protein was examined by monitoring the fluorescence pattern typical of amino acid residues according to Lakowicz (2006). Samples were prepared by adding PEPC (10 μg ml^{-1}) in 50 mM Tris-HCl [pH 7.3] and 10% glycerol (buffer A). The mixture was incubated at different temperatures for 45 min in a thermoregulated water bath. The fluorescence emission spectrum of each sample was recorded at room temperature with a spectrofluorimeter (Spec Fluoromax 3). The samples were excited at 280 nm and the emission spectrum measured between 300 and 400 nm using a 1 cm light path. Further details are given in the description of the results. Extrinsic fluorescence was monitored using 1-anilinonaphthalene-8-sulfonic acid (ANS).

Circular Dichroism (CD) Spectra

Circular dichroism (CD) measurements were recorded with a spectropolarimeter (Jasco J-810) using 1.0 cm path length cell and an average of 3 repetitive scans between 250 and 200 nm according to Micsonai (2015). The spectra were recorded with a scan speed of 20 nm min^{-1} and with a response time of 1s. The CD results were expressed as mean residue ellipticity (θ), according to Sievers (1978).

Results

The phosphorylation state and protein levels of PEPC were investigated to see if the temperature change affected them in any way. Since sunlight is known to promote PEPC's phosphorylation, leaf discs were subjected to light and dark treatments for comparison. In contrast to leaves that were dark-adapted, lighted discs had considerably greater PEPC phosphorylation levels. However, in leaf discs subjected to various temperatures, there was no appreciable change in the

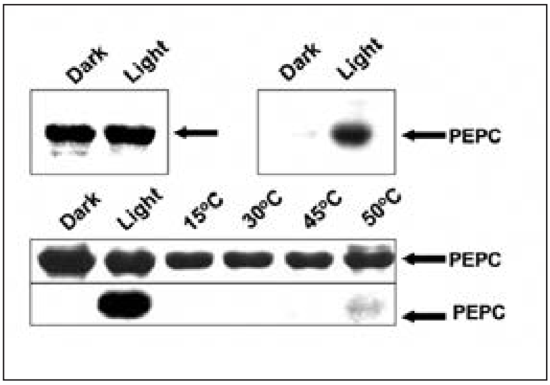

Figure 2: Illumination causes reversible changes in the activity and properties of PEPC in leaves of C_4 plants.

status of PEPC phosphorylation (Figure 4). According to a Western blot examination, the levels of PEPCprotein in the leaf discs of *A. hypochondriacus* were practically identical in the lighted, dark-treated control, and temperature-varying discs (Figure 2). Therefore, neither a change in temperature nor illumination resulted in a discernible shift in the amounts of PEPC protein.

It is probable that the presence of glycerol and/or PEG results in an oligomerization of PEPC resulting to an increase in the Vmax and alterations in other characteristics. Therefore, experiments using non-denaturing PAGE were created to measure the molecular size of PEPC in the presence of these suitable solutes. The nature of the protein was investigated on native PAGE after the purified enzyme had been treated for 45 minutes with PEG or glycerol (Figure 3). According to native PAGE analysis, cold temperatures tend to change the PEPC protein's equilibrium in favour of tetramers, whereas higher temperatures result in the loss of tetramers. Only a minor effect of glycerol was seen at higher temperatures.

Fluorescence emission spectra of PEPC purified from *A. hypochondriacus* were examined, using different concentrations of protein in the medium (Figure 4A). When the protein was excited at 280 nm, the emission of fluorescence had

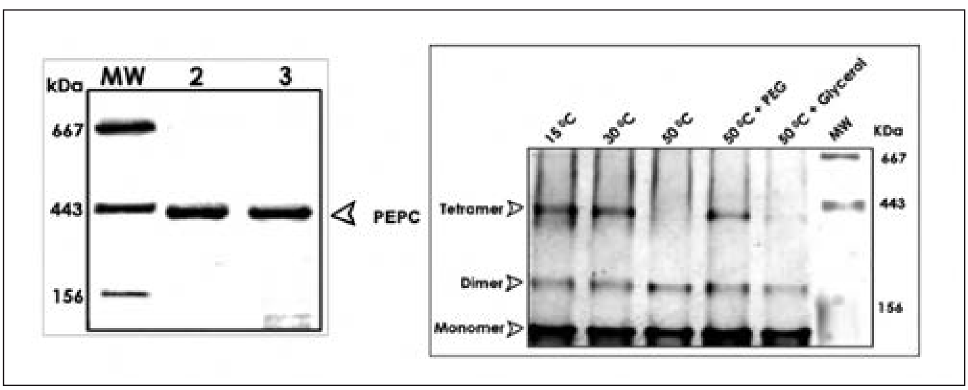

Figure 3: Effect of PEG or glycerol or both on the structure of PEPC during native PAGE.

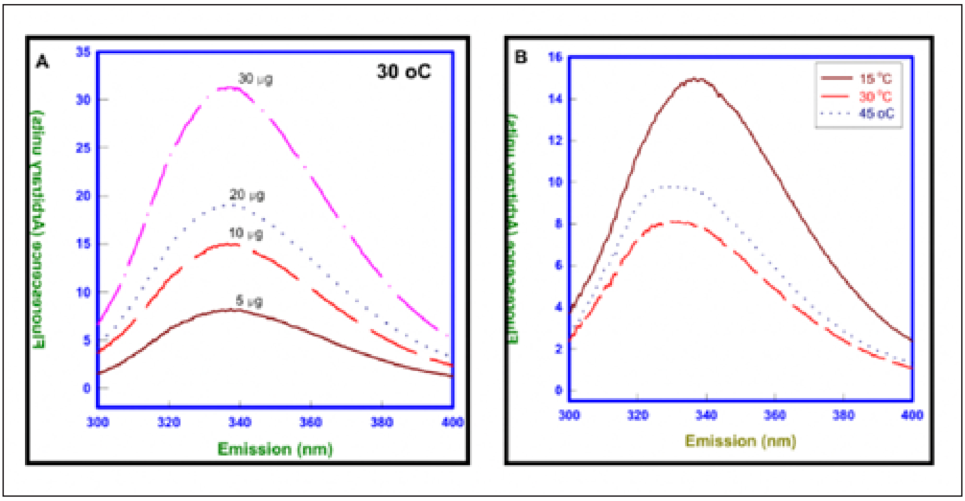

Figure 4: (A) Intrinsic fluorescence spectra of PEPC purified from A. hypochondriacus leaves after exposure to 30°C. for 45 min. Different concentrations of protein in the incubation medium were used. Excitation of protein was at 280 nm. (B) Intrinsic fluorescence spectra of PEPC purified from A. hypochondriacus leaves (10 μg PEPC/ml in buffer A, see "Materials and Methods") after exposure to different varying temperatures of 15°C., 30°C. or 45°C. for 45 min.

a maximum 335 nm. The intensity of fluorescence emission increased, as the concentration of protein was raised from 5 µg to 30 µg mL^{-1}. Further experiments were done with 10 µg mL^{-1} of PEPC protein. The intrinsic fluorescence spectra of PEPC decreased markedly with the increase in temperature from 15°C. to 45°C. (Figure 4B). In addition, there was a shift in emission maxima from 339 nm, to 335 nm and to 333 nm at 15°C., 30°C. and 45°C. respectively.

The pattern of fluorescence emission can also provide a measure of qualitative changes in the enzyme in response to the binding of malate or Glc-6-P. In the presence of L-malate, the competitive inhibitor and Glc-6-P, the allosteric activator of the enzyme PEPC exhibited a significant difference between the emission spectra at different temperatures was observed (Table 1). The signal around 340 nm was enhanced in presence of Glc-6-P at 15°C. whereas the intensity of fluorescence at 30°C. and 45°C. were almost similar. There was a progressive quenching of intrinsic fluorescence in presence of malate at all temperature tested.

The extrinsic fluorescence of PEPC (due to ANS) increased markedly as the temperature was raised from 15°C to 45°C (Figure 5). There was also a slight shift in the peak of emission from 454 nm at 15°C to 456 nm at 30°C and 459 nm at 45°C. L-malate, a competitive inhibitor of the enzyme, increased the extrinsic fluorescence of PEPC, while Glc-6-P, an allosteric activator, had no significant effect on the fluorescence (Figure 6).

Table 1: Effect of PEG-6000, urea, Glc-6-P or malate on the intrinsic fluorescence of A. hypochondriacus PEPC. The intrinsic fluorescence was measured as described under "Materials and Methods". The values in the parenthesis are % of that at 30°C. The enzyme was exposed to varying temperature in the absence or presence of 1.25% (w/v) PEG-6000, 3 M urea, 2 mM Glc-6-P and 0.5 mM malate and the characteristics of PEPC protein were determined.

Effector	Temperature		
	15°C (Low)	30°C (Moderate)	45°C (High)
	Intrinsic fluorescence (Arbitrary units)		
None (Control)	17.40 (129)	13.40 (100)	9.46 (70)
+ Malate	7.56 (110)	6.83 (100)	6.28 (92)
+ Glc-6-P	14.19 (272)	5.22 (100)	4.21 (80)
+ PEG-6000	29.48 (125)	23.58 (100)	21.44 (91)
+ Urea	7.12 (111)	6.41 (100)	4.27 (66)

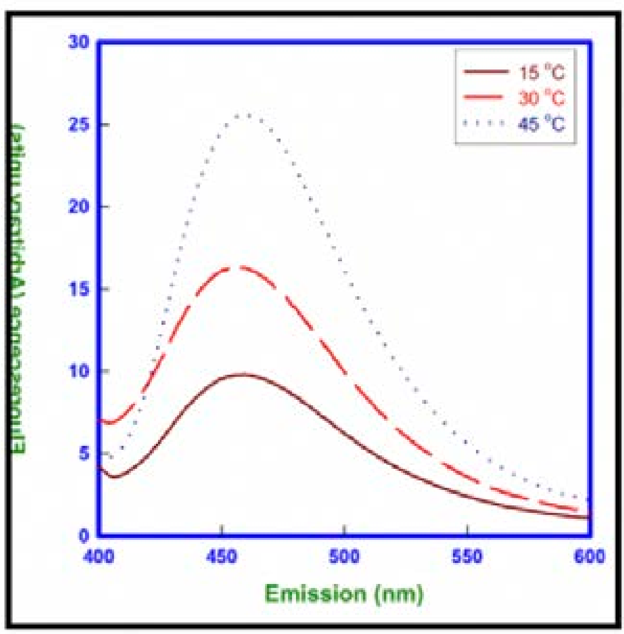

Figure 5: Extrinsic fluorescence spectra of PEPC purified from A. hypochondriacus leaves after exposure to different varying temperature.

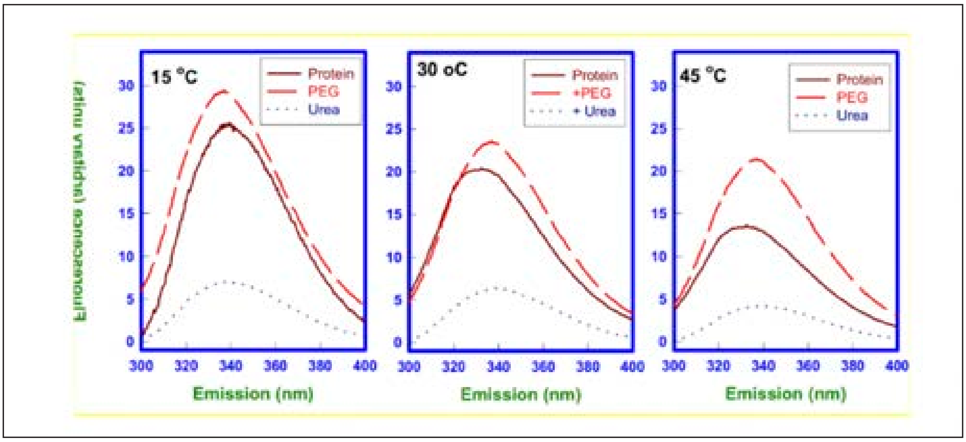

Figure 6: The effect of 1.25% (w/v) PEG-6000 and 3 M urea on the extrinsic fluorescence of PEPC from A. hypochondriacus, exposed to varying temperatures.

The far UV-CD spectrum of PEPC showed a broad negative band from about 210 to 220 nm, which is characteristic of a protein having α-helices. The mean residue negative ellipticity of PEPC decreased markedly as the temperature was raised from 15°C to 45°C. At 45°C, there was a shift in negative band at 227 nm. Estimates of helical content of the PEPC protein showed that the % of α-helices decreased with increase in temperature (Table 2). Similarly, there was an increase in β-sheet with increase in temperature.

Table 2: Secondary structure content of PEPC protein from Amaranthus hypochondriacus incubated at different temperatures for 45 min as indicated by the estimated percentages of α -helix, β -sheet and random coil from CD curves.

Temperature	α-helix	β-sheet	Random coil
	Percentage (%)		
15°C	80.7	6.82	12.4
30°C	47.2	9.47	43.3
45°C	28.3	11.8	59.9

Discussion

The results from this study demonstrate that a change in temperature can induce quite dramatic changes in not only the activity but also the malate sensitivity of PEPC in both C_3 and C_4 plants. The changes were caused by a short duration of exposure to temperatures and were reversible to a large extent. The temperature induced changes can therefore be physiologically relevant and important.

It may be argued that other factors may possibly affect the physiology of leaf discs at different temperatures for e.g., concentration of dissolved CO_2, oxidative stress, or induction of heat shock proteins. The marked reversibility temperature effects of PEPC support the concept that leaf discs are ideal to simulate the conditions *in vivo*.

The regulation of PEPC is achieved by posttranslational modification of the enzyme, by phosphorylation of a serine residue near the N-terminus of PEPC (Rajagopalan et al., 1994; Chollet et al., 1996; Vidal and Chollet, 1997). The phosphorylation of the enzyme leads to an increase in the activity of enzyme and decrease in the malate sensitivity. The changes induced by rise in temperature, namely the increase in the activity and decrease in the extent of malate inhibition are quite similar to the changes effected during light activation of PEPC. It is therefore, quite possible that there is a change in phosphorylation status of PEPC on exposure to temperature. However, our experiments rule out the possible role of phosphorylation of enzyme in temperature effects of PEPC (Figure 5). This is the first report that the temperature induced changes in PEPC of C_4 species is independent of phosphorylation.

An attractive and alternative possibility is the change in the conformational status of the enzyme. PEPC is very active when it is in a tetrameric shape, while its activity and malate sensitivity decreases when the enzyme dissociates into a monomer or dimer (Shi et al., 1981; Walker et al., 1986; Willeford et al., 1990). Temperature may affect the oligomeric status of the enzyme PEPC. Rise in temperature causes the aggregation of PEPC in case of C_4 and dissociation in case of CAM (Wu and Wedding, 1987). Cold/chilling may make the enzyme to dissociate from active tetrameric shape to less active dimers or monomers. However, this is not well corroborated in case of C_4-PEPC (Walker et al., 1986).

McNaughton et al. (1989) reported that changes in oligomerization state of PEPC may not be related to malate sensitivity and light induced changes. It is possible that other types of conformational changes would still occur, for e.g. in the hydrophobic microenvironment of the protein.

Fluorescence is a useful tool for monitoring the conformational changes in protein molecules. Proteins exhibit significant intrinsic fluorescence, mainly due to their aromatic amino acids: tryptophan, tyrosine and phenylalanine. The fluorescence of tryptophan is influenced by several factors such as pH, temperature and the solvent. Tryptophan in non-polar solvent exhibits maximum emission at

320 nm, whereas in aqueous environment, emission maxima is 355 nm. The fluorescence intensity decreases on protonation of tryptophan and is also quenched by neighbouring acidic groups as well as high temperature (Lakowicz, 1983).

The PEPC protein from *A. hypochondriacus* exhibited high intrinsic fluorescence. The peak of fluorescence at 335 nm suggests that most of the protein's intrinsic fluorescence is possibly due to tryptophan residues. The marked changes in PEPC with temperature in the intrinsic (as well as the extrinsic) fluorescence demonstrate that marked changes are occurring in the tertiary structure of the enzyme. A similar phenomenon was found in PEPC of *Rhodothermus obamensis* during thermo-denaturation at different temperatures (Takai et al., 1997). The *Rhodothermus* PEPC showed a decrease in intrinsic fluorescence during denaturation at higher temperature, and a shift in the peak (Takai et al., 1997). A decrease in the intrinsic fluorescence of protein was also noticed in the case of GAPDH of *Thermatogo maritime* and RNase from *Rhizospora stolonifer* (Wrba et al., 1990; Desphande et al., 2003).

The nature of changes in protein could be compared and ascertained by using factors, which were earlier used for studies on protein conformation. For example, PEG-6000 is known to promote the aggregation of several enzymes, including PEPC, pyruvate kinase and FBPase (Huber and Sugiyama, 1986; Podestá and Plaxton, 1993; Hodgson and Plaxton, 1995). While urea, at concentrations of 3 M or higher, dissociates the proteins effectively (Encinas et al., 1998; Encinas et al., 2002). Thus, the increase in intrinsic fluorescence by PEG is an indication of aggregation of the protein. In contrast, the decrease in intrinsic fluorescence in presence of urea suggests the dissociation/denaturation of the enzyme. By drawing an analogy, the increase in intrinsic fluorescence of PEPC at 15°C, and the decrease at 45°C indicates that PEPC gets dissociated at warm temperature (e.g., 45°C) and tends to be aggregated or oligomerized at cold temperatures (e.g., 15°C).

ANS presents an emission maximum at 524 nm that decreases to 462 nm in presence of C_4 PEPC, indicating binding of the probe to the protein. This is similar to the previous results with PEP carboxykinase from *E. coli* (Encinas et al., 1998) or *Saccharomyces cerevisiae* (Encinas et al., 2002). With a rise in temperature from 15°C to 45°C, there was an enhancement of fluorescence intensity and the shift in emission maxima of ANS. Such increase in extrinsic fluorescence suggests an increase in the binding of dye due to exposure of hydrophobic regions to the surface on thermal denaturation. Similarly, Deshpande et al. (2003) observed that the blue shift in emission and enhancement of fluorescence intensity of ANS at

70°C in RNase from *Rhizopus stolonifer* indicated the presence of solvent exposed hydrophobic surfaces as a result of heat denaturation.

The reversibility of changes, induced in intrinsic fluorescence of PEPC protein emphasizes the physiological significance of temperature-induced changes in PEPC. According to Schultes and Jaenicke (1991), the changes occurring in GAPDH from *Thermatoga maritime* are reversible, which exhibits intrinsic thermostability with denaturation temperature. Similar studies in the enzyme lysozyme show that the conformational changes are reversible (Chryssomallis et al., 1981). However, 3-isophenylmalate dehydrogenase from *Thermus thermophillus* showed irreversible changes (Imada et al., 1995). PEG promotes the tighter folding in the case of C_4 PEPC, which is in agreement with results obtained with castor oil seed FBPase (Hodgson and Plaxton, 1995). By contrast, denaturation of PEPC with 3 M urea caused the fluorescence emission intensity to substantially decrease, as the temperature increased without any pronounced shifts in its emission maximum. However, in case of yeast PEP carboxykinase, the presence of urea caused not only a quenching of the intrinsic fluorescence, but also a shift in the emission maxima towards red (Encinas et al., 2002). Similar changes were obtained in the presence of urea-induced unfolding of tetrameric PEP carboxykinase from *E. coli* and *Saccharomyces cerevisiae* (Encinas et al., 1998; 2002).

Far UV circular dichroism reflects the secondary structure of a protein and is hence used frequently as a tool to monitor protein conformation. It was therefore very useful to study the CD-spectra of PEPC subjected to different temperatures, to understand the nature of changes in protein on exposure to temperature. The crystal structure of maize PEPC (C_4 form) confirmed the tetrameric nature of protein, made of dimers. The monomer contains eight standard β-barrel with an abundance of α-helices (Matsumura et al., 2002). According to Kai et al. (2003) *E. coli* PEPC monomer consists of an eight standard β-barrel and approximately 40 α-helices comprising of 65% of the polypeptide, whereas β-strands comprised only 5%. The secondary structure of *Zea mays* PEPC is almost similar to that of *E. coli* PEPC, except for certain regions (Kai et al., 2003). The CD-spectra of C_4 PEPC protein from *A. hypochondriacus* shows characteristic dual peak at 208 and 222 nm suggesting that the major part of protein is in α-helical conformation. Quantitative analysis of the CD spectra revealed that the PEPC of *A. hypochondriacus* too contained significant proportion of α-helices, while the β-sheets were $<10\%$, at 30°C. The use of CD-spectra has revealed interesting changes in several proteins with factors such as temperature. For example, rapid changes in magnitude and shape of CD- spectra in case of barstar with increasing temperature, suggested marked changes in protein under thermal denaturing conditions (Nölting et al., 1997). However, Kamal and Behere (2002) observed that the heat induced denaturation was rather mild in case of soyabean peroxidase. In another interesting study, recombinant PEPC from *Thermus* species was subjected to limited proteolysis and the generated major fragments were used for CD spectral analysis. Peptide cleavage had no substantial effect on the PEPC quaternary secondary structure and thermostability (Nakamura et al., 2002). The CD spectrum of PEPC from *A. hypochondriacus* showed a pronounced dual peak, after the pretreatment at 15°C, indicating a marked folding of the protein with a rigid α helical conformation. In contrast, when pretreated at warm temperature, 45°C, the curve showed a significant reduction of dual peak, thus revealing a marked reduction in α-helicity (Figure 4; Table 1). These results are similar to the findings of Nölting et al. (1997), who observed a significant loss of α-helix and increase in random coil with thermal denaturation of barstar protein. However, Nölting et al. (1997) employed subzero temperatures, while we recorded an increase in α-helicity of PEPC at even 15°C (compared to that at 30°C). PEG-6000 and urea are known to induce aggregation and dissociation of proteins: for e.g., barstar and yeast PEP carboxykinase (Nölting et al., 1997; Encinas et al., 2002). The CD-spectra of PEPC showed an increase in negative ellipticity and dual peak nature, in presence of PEG suggesting that PEG-6000 induced the protein to attain a high α-helical conformation. In contrast, 3 M urea caused a marked decrease in the a-helicity and increased random coil percent of PEPC, indicating a complete disruption of secondary structure of the protein. Further studies were extended to check the effect of Glc-6-P (an allosteric activator) and malate (a competitive inhibitor). The negative ellipiticity of CD-signal of PEPC was enhanced in presence of Glc-6-P irrespective of temperature: 15°C, 30°C or 45°C. On the other hand, malate caused limited disruption of secondary structure and the effect of malate was more pronounced at 30°C and 45°C than that at 15°C (Figure 5). Matsumura et al. (2002) reported that in the vicinity of negative affector binding site in *Zea mays* PEPC structure, large conformational changes are observed compared with the aspartate bound T-state. The present study emphasizes that the PEPC protein undergoes marked conformational changes in its secondary structure, after treatment at different temperature. Changes can also be seen in the presence of compatible solutes like PEG-6000 or urea (a protein denaturant), or effectors like Glc-6-P (allosteric activator) and malate (competitive inhibitor). The α-helicity of PEPC is stabilized at cold temperature and destabilized at warm temperature.

Conclusions

During illumination, the phosphorylation of the protein leads to an increase in the activity of PEPC and decrease in the malate sensitivity. The changes induced by rise in temperature (increase in the activity and decrease in the extent of malate inhibition) are quite similar to the changes during light activation of PEPC. However, our results indicate that the phosphorylation is not the main reason for the temperature effects on PEPC. Non-denaturing PAGE of purified PEPC showed the existence of three different forms with proportionally increasing molecular weight: monomer, dimer and tetramer. •Cold temperature tends to shift the equilibrium of PEPC protein towards tetramer. The presence of PEG and/or glycerol resulted in predominance of tetramer. PEG-6000 and urea are known to promote aggregation and dissociation of the protein, respectively. Thus, the PEPC protein is expected to unfold in the presence of urea and fold in the presence of PEG-6000. The increase in the extrinsic fluorescence of PEPC suggests that the increase in the binding of the dye, obviously due to the unfolding of protein, appears to increase at warm temperatures. Urea of 3 M induced almost complete disruption of secondary structure of the protein. However, PEG increased the α-helical content, and obviously stabilized the protein.

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Journal of Arts, Science and Technology

Host Preference, Impact of Host Transfer, and Insecticide Susceptibility Among *Aphis gossypii* Group (Order: Hemiptera) in Jamaica

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Abstract

Integrated pest management strategies for Aphis gossypii Glover 1877 (Hemiptera, Aphididae) require application of the knowledge of its biology and ecology, especially as it relates to its host relations. It is one of the world's most polyphagous agricultural pests and has developed resistance to many commonly used insecticides (Blackman and Eastop 2000; Godfrey and Fuson 2001). This study was designed to examine the level of insecticide susceptibility amongst Aphis gossypii in Jamaica while exploring fecundity and colony growth as a measure of host preference and host transfer success. Aphids collected in the field were acclimatized on selected host plants Capsicum chinense Jacquin 1776, Cucumis sativus Linnaeus 1630, Gossypium hirsutum Linnaeus 1751 and Abelmoschus esculentus (L.) Moench 1794 for three generations. Fecundity and colony size were then documented daily. The same measures were used after aphids were transferred among the hosts as a measure of preference. Mortality and fecundity were determined after the acclimatized aphids were exposed to varying concentrations of four common insecticides: Actara®, Diazinon™, Karate Zeon® and Pegasus®. Host preference results indicated that, over a 15-day period, Aphis gossypii reached its largest colony size on cotton ($\bar{x}$ 14). Host transfer experiments were all significantly different with the most significant occurring between transfers from pepper to cucumber ($p < 0.05$) which indicates that host transfer success is highly probable once suitable hosts exist within an intercropping sys-

tem. Insecticides when ranked from most lethal to the least lethal were Karate Zeon®, Actara®, Pegasus® then Diazinon™. The highest LC50 values were obtained for aphids on cotton and pepper with Pegasus® and for those on cucumber with Diazinon™. Resistance was not found; survivors of insecticide treatments can successfully repopulate host plants but have a lower growth rate. The growth rate of aphid colonies treated with insecticides was on average 80% less than that of untreated aphids.

Keywords: Aphis Gossypii, Host-Plant Preference, Colonization Sequence, Host Transfers and Insecticide Susceptibility

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Introduction

Aphis gossypii (cotton aphid) is a polymorphic, polyphagous, sap feeding insect pest that transmit plant pathogens such as viruses. It has pest status in many locations such as Asia and North America and has developed reduced susceptibility to many active ingredients commonly used in insecticides such as 2-isoproppyl-6-methyl-4-pyrimidinyl (Saito, 1991; Furk and Hines, 1993; Ninsin, 2017; Mota-Sanchez and Wise, 2020), cyhalothrin-lambda (Herron, Powis, and Rophail, 2000) and diafenthurion (Mota-Sanchez and Wise, 2020).

In tropical regions, like the island of Jamaica, only anholocyclic life cycles have been recognized (Dixon, 1974; Hall, 2005; Blackman and Eastop, 2006) and we have used their family group names. Bodenheimer & Swirski's recent book (8. Two morphs, the viviparous aptera and alata are found in Jamaica and they may vary in colour and size (Ebert and Cartwright, 1997; Hall, 2005b). This species is regarded as a group by many researchers as it is biologically diverse and taxonomically challenging (Blackman and Eastop, 2006; Lagos-Kutz et al., 2014)1984, 1994, 2000.*Aphis gossypii,* as a pest, directly damages its host plant by extracting sap (sucking) and indirectly damages it by transmission of viruses, ultimately affecting the yield of the host (Blackman and Eastop, 2000). In Jamaica there have been outbreaks of viral plant pathogens such as the *Papaya ringspot virus* (Tennant, Ahmad, and Gonsalves, 2005) and *Citrus tristeza virus* (Fisher, Tennant, and McLaughlin, 2010)885 that were transmitted by *Aphis gossypii*.

Aphis gossypii is extremely polyphagous and linked to over 700 secondary host plants world-wide (Bass et al., 2015) including over 200 plants in more than 60 families in Australia (Waterhouse & Sands, 2001); over 209 plants in more than 40 families in Florida (Bass et al., 2015; Capinera, 2018); and fifty-eight species in 28 families in Jamaica (Hall & Garraway, 2013). *Gossypium* sp. is a primary host in some areas where the holocyclic life cycle exists. Among the host are economically significant host plants including pepper, eggplant, watermelon, cucumber, and hibiscus. The wide range of non-economic host may serve as a reservoir for colonisation of important crops. Host-associated races and biotypes have been identified in different parts of the world by Carletto et al., (2010), Agarwala and Choudhury, (2013) and Vanlerberghe-Masutti, Chavigny, and Fuller, (1999). Hu et al. (2017) reported that cucurbit and cotton specialized biotypes existed and found that *A. gossypii* from cotton and cucumber preferred their initial/original host plant.

Factors affecting host choice has been investigated. Host preference may be linked to a combination of environmental stimuli and genetics (Komazaki and Toda, 2008). The genetical basis involves intricate interactions of many genes (Komazaki and Toda, 2008), especially chemosensory genes (Gu et al., 2013; Cao et al., 2014; Kang et al., 2017). Nutritional secondary metabolites in host plants are known to affect polyphagous preference and performance of *Aphis gossypii* (Deguine and Hau, 2001). The environmental cues include host plant quality, crowding and predation risk (Ralec et al., 2010; Schmidtberg and Vilcinskas, 2016).

In natural environment a polyphagous aphid can migrate from one host to another due to overcrowding, wind, heavy rainfall, and ant-attendance (Slosser, Pinchak, and Rummel 1989; Ghovanlou 1976; Hosseini et al., 2017). In some parts of the world like in Connecticut and USA, it was shown that A. gossypii living on Malvaceae (*Hibiscus syriacus*) and Bignoniaceae (*Catalpa bignoniodes*) could easily be transferred to and develop on cotton, but hardly on cucumber Kring (1959). While aphids from cucumber in Mississippi, USA produce few to no offspring when transferred to other host plants (Williams, 2003). The differences in performance on different hosts has led to the suggestion that genotypic differences exist within A. gossypii populations with respect to the host plant (Brèvault et al., 2011). Exploring host transfers and genetic analysis can elucidate the host preference of Aphis gossypii; its ability to grow larger and become more fecund on certain host plants as opposed to others (Blackman and Eastop, 2006; Miller and Foottit, 2009). Host plants that are preferred will have lower mortality, higher fecundity, and longer lifespans.

Insecticide application is the primary means of treating aphid infestations. In Jamaica, the main chemicals used to treat *Aphis gossypii* are: Karate Zeon®, Actara®, Pegasus® and Diazinon™. *Aphis gossypii* has develop resistance to the active ingredients of commonly used insecticides in some parts of the world such as California, USA (Jones, 2004; Vorwerk, 2007)Aphis gossypii (Glover and China (Kaur and Garg, 2014). Godfrey and Fuson (2001) pointed out that *Aphis gossypii's* speedy acclimation to major insecticide groups is linked to their parthenogenesis. If one aphid develops the slightest resistance to an insecticide, this resistance will be passed on to subsequent generations since all aphids within a population have identical genotypes until there is a sexual generation (Blackman and Eastop, 2006).

To facilitate integrated pest management, it is imperative to understand the biology of the aphid and its host preference. Preliminary work in Jamaica has indicated differences in biology and host preference within the species (McDonald et al., 2003; Hall, 2005a). However, specific details of fecundity, colony growth, host preference, distribution, and insecticide resistance of *Aphis gossypii* were unknown. This work was conducted to determine host preference of *Aphis gossypii* as indicated by colonization sequence, fecundity, colony size and host transfer success as well as its susceptibility to four commonly used insecticides.

Materials and Methodology

Preparation of Host Plants

Seedling trays containing sterilized Evergreen™ Horticultural Products Seedling & Cutting Mix soil were used to sow seeds of *Capsicum chinense* Jacquin 1776 (pepper), *Cucumis sativus* Linnaeus 1630 (cucumber), *Gossypium hirsutum* Linnaeus 1751 (cotton), and *Abelmoschus esculentus* (L.) Moench 1794 (okra), under glasshouse conditions. Seedlings were watered daily and used when they had at least 5 leaves.

Determination of Colonization Sequence in the Field

Sixteen seedlings each of cucumber, pepper, and cotton with four true leaves were inter-planted in 4 x 4 metre field plots at the UWI Mona Department of Life Sciences' Botany Garden. Once colonies were founded, daily counts were made until there were 100 aphids.

Determination of colony growth and fecundity in the glasshouse

Aphids from the field were acclimatized for three generations on the same host species in the glasshouse. Then, third instar aphids were transferred to leaves of a caged, potted host individual. Colony size was documented daily for 15 days. Fecundity, reproductive span, and lifespan were determined from host individuals from which nymphs were removed after birth.

Determination of colony size after host transfer

Third instar *Aphis gossypii* were transferred individually to five leaves on three replicate potted seedlings of the original host plant and of the other host plants. The number of aphids in each colony was counted for 15 days.

Determination of insecticide susceptibility

100 ml of a 0.01 %, 0.05 %, and 0.10 % dilution of Actara®, Diazinon™, Karate Zeon® and Pegasus® were prepared with distilled water. A Potter's Precision Laboratory Spray Tower was used to apply 1.0 ml of each dilution to Petri dishes containing ten adults from colonies acclimatized on cucumber, hot pepper, and cotton for three generations. Ten replicas were done for each host for each concentration for each insecticide and the control (distilled water). The number of aphids that died was recorded after eight hours (as a proportion of the total number of aphids in the Petri dish). The pooled survivors of each concentration of each insecticide were transferred to new plants of their respective host species and the colony size determined after 15 days. Average daily colony growth rate of survivors of 0.1 % insecticide was determined.

Analysis

Microsoft Excel™ 2019 was used to format the data for analysis. Shapiro-Wilk's test for normality Levene's test for equal variance, Kruskal-Wallis test for comparing groups on a dependent variable and the ANOVA's test for analysing the difference between the means of more than two groups were performed using R™ (R Studio Version 3.5.3). Probit analyses were performed to determine LC_{50} and LC_{95} of the insecticides using Polo-PC by Leora Software.

Results

Colonies were established on cotton and got to 100 individuals before initiation on the other host plants in the field (Table 1). The order of preference was cotton, pepper, cucumber then okra (Tables 1 and 3). *A. gossypii* on cotton had the highest fecundity (Figure 1) and the longest reproductive span and lifespan (K= $p < 0.05$) (Table 2) and the largest colony sizes (Figure 2). The colonies on all host plants were affected by the time of year (F= $p < 0.5$) (Figure 2).

The number of new aphids added to the colony of the four host plants every three days indicated that on average, fecundity was highest during days 8–11 (Figure 1). Transfer to another host species influenced the size of colonies (Figure 3). The aphids performed best when the recipient individual was of the same species as the original host plant (K= $p < 0.05$).

Insecticides ranked on lethality are Karate Zeon®> Actara®> Pegasus® > Diazinon™. The highest LC_{50} values were obtained for aphids on cotton and pepper were with Pegasus® and for those on cucumber with Diazinon™ (Table 3). Survivors of insecticide treatments can successfully repopulate their hosts, but colony growth can fluctuate (Table 4). The colony growth rate of non-treated aphids is significantly slower (K= $p < 0.05$) than that of survivors (Table 5).

Table 1: Colony Initiation and growth timelines for Aphis gossypii in the field

	Mean Number of Days for Colony Initiation and Growth to 100 aphids	
Host Plant	**Initiation**	**Growth to 100**
Cotton	12.3	23.3
Cucumber	25.6	44.7
Pepper	29.0	41.3
Okra	35.6	62.7

Table 2: Mean Lifespan and Reproductive Span of A. gossypii in the glasshouse

	Cotton	Cucumber	Pepper	Okra
Lifespan	20.67 ± 0.53	19.87 ± 0.64	19.27 ± 0.52	18.07 ± 0.72
Reproductive Span	25.27 ± 0.53	24.93 ± 0.66	24.27 ± 0.52	23.73 ± 0.59

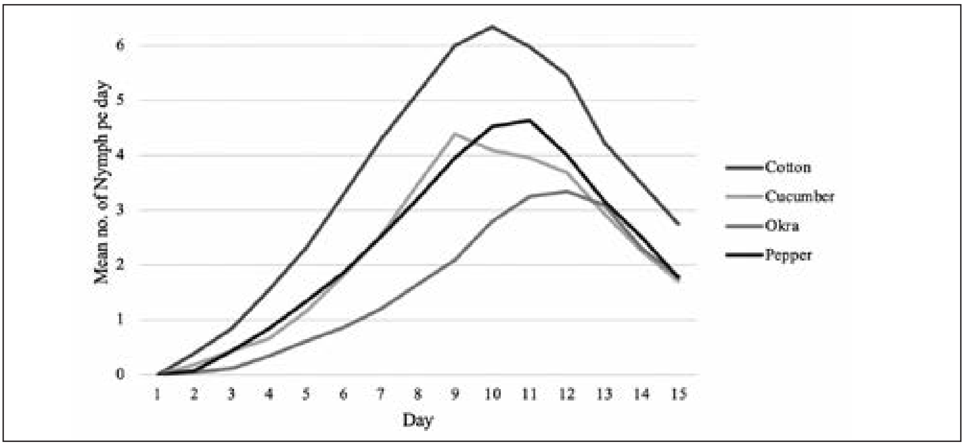

Figure 1: Mean Fecundity of A. gossypii in the glasshouse

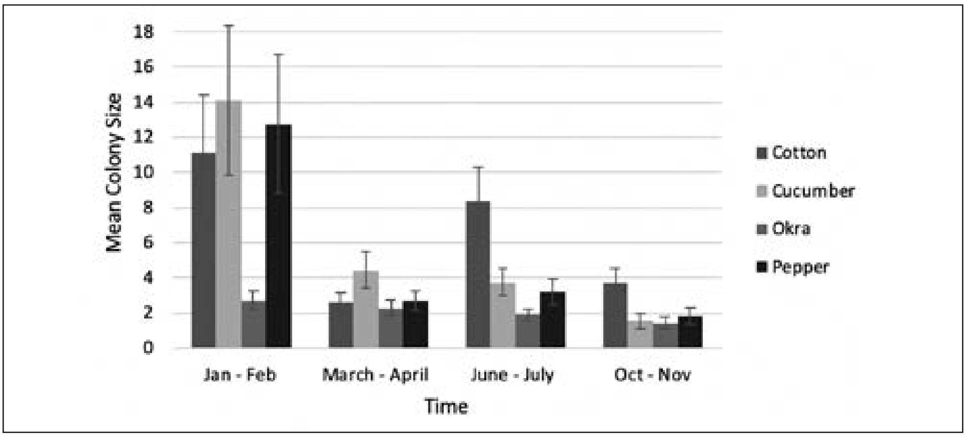

Figure 2: Total Mean Colony Size Aphis gossypii in the glasshouse after 15 days

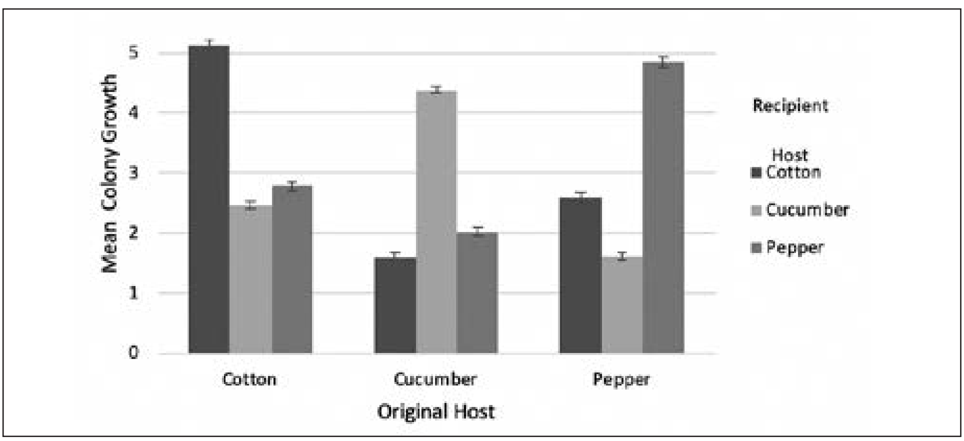

Figure 3: Mean Colony Size of A. gossypii after 15 days Recipient Host after Transfer from Cotton, Cucumber and Pepper in the glasshouse

Table 3: Eight-Hour Probit Analysis of Insecticide Bioassay with Cotton, Cucumber and Pepper

Insecticide	Host Plant	Lethal Concentration (%)		95% Fiducial Limits	Slope
Actara®	Cotton	LC_{50}	0.066	0.027 – 0.126	0.545 ± 0.092
		LC_{95}	69.198	14.536 – 1385.690	
	Cucumber	LC_{50}	0.052	0.009 – 0.141	0.861 ± 0.103
		LC_{95}	4.268	1.008 – 274.507	
	Pepper	LC_{50}	0.021	0.001 – 0.064	0.691 ± 0.101
		LC_{95}	4.942	1.030 -669.725	
Diazinon™	Cotton	LC_{50}	0.154	0.041 – 0.442	0.496 ± 0.090
		LC_{95}	318.781	23.190– 963274.880	
	Cucumber	LC_{50}	0.212	0.060 – 0.717*	0.390 ± 0.089
		LC_{95}	3491.9	105.38 – 3.718E+8*	
	Pepper	LC_{50}	0.028	0.003 – 0.080	0.589 ± 0.095
		LC_{95}	17.325	2.940 – 2768.221	
Karate Zeon®	Cotton	LC_{50}	0.026	0.001 – 0.081	0.639 ± 0.098
		LC_{95}	9.612	1.635 – 3698.550	
	Cucumber	LC_{50}	0.0005	–	0.861 ± 0.282
		LC_{95}	0.0400	–	
	Pepper	LC_{50}	0.0008	0.0000 – 0.0030*	1.108 ± 0.430
		LC_{95}	0.0233	0.0109 – 0.0594*	
Pegasus®	Cotton	LC_{50}	0.226	0.111 – 0.458	0.508 ± 0.091
		LC_{95}	392.670	52.526 – 32254.563	
	Cucumber	LC_{50}	0.033	0.008 – 0.074	0.798 ± 0.103
		LC_{95}	3.813	1.168 – 49.264	
	Pepper	LC_{50}	0.043	0.018 – 0.080	0.621 ± 0.095
		LC_{95}	19.117	5.786 – 158.848	

Note: An asterisk (*) indicates a 90% rather than a 95% fiducial limit.

Table 4: Colony Sizes of Aphids Surviving Varying Concentrations of Insecticides

	Cotton (No. of aphids)		Cucumber (No. of aphids)		Pepper (No. of aphids)	
Insecticide Conc (%)	**Survivors**	**Day 15**	**Survivors**	**Day 15**	**Survivors**	**Day 15**
Actara® 0.01	45	15	21	3	35	2
Actara® 0.05	15	166	3	115	5	0
Actara® 0.1	25	89	3	22	7	0
Diazinon™ 0.01	37	105	26	84	22	43
Diazinon™ 0.05	32	198	27	68	23	174
Diazinon™ 0.1	24	98	15	36	12	36
Karate Zeon® 0.01	41	184	3	11	3	13
Karate Zeon® 0.05	22	190	1	10	0	–
Karate Zeon® 0.1	35	170	0	–	0	–
Pegasus® 0.01	64	200	28	140	34	171
Pegasus® 0.05	40	94	19	96	31	178
Pegasus® 0.1	44	110	17	34	15	41

Table 5: Average Colony Growth Rate of Survivors of 0.10 % Insecticide Treatment per Day

	Growth rate per aphid per day				
Host Plant	**Actara®**	**Diazinon™**	**Karate Zeon®**	**Pegasus®**	**Untreated**
Cotton	0.24	0.27	0.32	0.16	5.1
Cucumber	0.49	0.16	–	0.13	5.2
Pepper	0.00	0.20	–	0.18	4.4

Discussion

All hosts were colonized successfully by *Aphis gossypii,* but cotton was preferred both in the field and glasshouse. This may be due to the biotypes and host races present (Lombaert et al., 2009; Hu et al., 2017)pests are submitted to strong human-imposed selective pressures to which they sometimes adapt rapidly, either through selection of genotypes resulting from mutation and/or recombination events, or through phenotypic plasticity. Understanding how insects respond to such selective pressures is of great importance for sustainable pest management strategies, such as the use of resistant plants. In this study, we investigated the genetic and phenotypic variability of anholocyclic Aphis gossypii Glover (Hemiptera: Aphididae. Even hosts with less successful colonies, like okra, can serve as reservoirs for populating each other (within an intercropping system) based on migration due to overcrowding, heavy showers, high wind, or ant-attendance (Slosser, Pinchak, and Rummel, 1989). When aphids are transferred to a host plant of another species, colony development is slower than when they are transferred to host plants of the same species. In addition to difference in preference possibly due to biotypes, the plants' nutritional contents and secondary compounds vary, so aphids do require time to adapt to a new host plant after the transfer.

Management of *Aphis gossypii* infestations requires vigilance in the early stages of colony development. The fecundity increases exponentially between day 3 and 12, and daily nymph production on average exceeds four. The aphid's reproductive span is almost 80% of their lifespan (Table 2) and telescoping of generations results in the offspring reproductive span overlapping that of their parent. The host and the time of year also impact the colony sizes (Figure 2) with cotton tending to have larger colonies, especially in the earlier months of the year.

Many small farmers are unaware of core principles that guide effective usage of insecticides (dosage, frequency, application and safety) and focus solely on trying to eradicate the current infestation and increase their profit (Singh, 2012; Sun et al., 2019). This is also supported by (Bass et al. 2015 who argues that mismanagement will result in additional resistance. The aphids here, regardless of host, were most susceptible to the highest concentration excepting for that of Karate Zeon®. An increase in Karate Zeon® concentration above 0.05% had no further effect on mortality. Actara® was the next most lethal insecticide with concentrations of 0.10 % resulting in 50% mortality. George, Rao, and Rahangadale (2019) found that neonicotinoid groups (Actara® – thiamethoxam) were most effective in causing mortality amongst *Aphis gossypii*.

There was no significant difference in the susceptibility of the aphids from different hosts to the different brands of insecticide except for those acclimatized on cucumber (Table 3). Perhaps this may be linked to the different nutritional composition of cucumber (Ma et al. 2019) and its association with the different cucumber biotypes (Wang et al., 2016; Dai et al., 2018; Zhang et al., 2018; Ma et al., 2019)Aphis gossypii Glover (Hemiptera: Aphididae. Pan et al. (2018) suggested that metabolic factors such as UDP-glycosyltransferases (UGTs) may be linked to the increased development of insecticide resistance seen amongst sucking insect pest like *Aphis gossypii* to Actara® – thiamethoxam.

The LC_{95} values obtained, however, were all relatively high. The high LC_{95} would suggest a high level of resistance, but since it is coupled with low LC_{50}

values this was not the case. For resistance to be deemed present both the LC_{50} and LC_{95} values would have to be high (Elbert and Nauen, 2004; Miller, Tindall, and Leonard, 2010; Hagstrum and Subramanyam, 2016).

Based on the prevalence of insecticide usage, most aphids will come into contact with a low concentration of these insecticides at some point (Bass et al., 2015). Thus, with low LC50 values, but high LC95 values, mortality is only guaranteed when aphids encounter extremely high concentrations. Thus, at least 50% of the population will survive and still be fit to produce offspring. Therefore, these offspring can then, over time, begin to develop even further resistance as the generations progress (Horowitz, Denholm, and Nichols, 2006; Miller, Tindall, and Leonard, 2010). This may be enhanced by further contact with low concentrations of insecticides (O'Brien and Graves, 1992; Mullins, 1993; Gore et al., 2013; Seyedebrahimi et al., 2016).

In Jamaica, *Aphis gossypii* is fully parthenogenetic (Hall, 2001) so if even 5 % of the population survived after being treated with high LC_{95} concentration(s), a new population can develop. Thus, the probability of developing resistance remains, as seen when survivors of the insecticide trials were able to successfully establish new colonies (Table 4), albeit with reduced growth rates (Table 5).-

All the insecticides used in this study to treat *Aphis gossypii*, in one way or another, was effective (RADA 2018; PCA 2020). For optimal results, it is recommended that agriculturalists use either Actara® or Karate Zeon® because not only are these insecticides effective in initially killing at least 50 % of the population on a host plant (while having lower pollution impact), but they also have the least successful survivor colonies.

Conclusion

Capsicum chinense, Cucumis sativus, Gossypium hirsutum and Abelmoschus esculentus are among the host for *Aphis gossypii* in Jamaica. The aphid was found to produce larger colonies sizes, have longer lifespans and longer reproductive spans on *Gossypium hirsutum*. The fact that aphids can be transferred among different hosts species successfully has implications for polycultural cultivation. Karate Zeon® was the most lethal insecticide against *Aphis gossypii* but all four insecticides tested had varying levels of success on the different hosts. Resistance was not found but where aphids survived, they were found likely to re-establish colonies. This has implications for the insecticide treatment regime that needs to be employed for the management of the aphid with insecticides. An integrated pest management program involving monitoring for colony initiation on preferred hosts and for survivors after insecticide treatment could be effective.

Acknowledgements

We thank the University of the West Indies Mona Research and Publication Grant and the New Initiative Grant. Special thanks to Mr. Otis Francis, the Crop Production Manager of Imagination Farm and Mr. Gavin Campbell for all their assistance.

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A Baseline Assessment of Lionfish (*Pterois spp.*) Population Dynamics, Distribution, and Diet Within the Montego Bay Marine Park, Jamaica, in 2018

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Abstract

Lionfish (Pterois spp.) are an Indo-Pacific species of venomous marine fish that have successfully invaded the waters of the Atlantic. Their generalist nature allows them to occupy and thrive in a multitude of habitats and prey on a myriad of organisms. Invasive Lionfish pose deleterious effects to an environment's overall health and functioning, by the (over)consumption of keystone species, such as herbivorous Parrotfish. During the calendar year of 2018, 117 recorded Lionfish sighting sessions were logged, and 902 specimens were captured across >40 distinct sites. Meta-data from sessions and dissection data of specimens were compiled to project the general distribution, size, maturity, and the overall diet of Lionfish within the Montego Bay Marine Park. Length-Weigh frequency of specimens were graphed. Lionfish occupied 7 habitat types: (low-, medium-, and high-profile) coral reefs, (low-profile) artificial reefs; sand patches, rock/rubble patches, and seagrass patches. Furthermore, certain sites were revisited, to determine the effectiveness of consistent and interval culling efforts. The data gathered will help identify attributes of the population within the sites, and aid in further management strategies.

Keywords: Lionfish, Population Dynamics, Diet Composition and Stomach Content Analysis

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Introduction

Lionfish Background

Pterois is a genus of venomous, ornate ray-finned marine fishes, belonging to the family Scorpaenidae. They are native to the Indo-Pacific region (Côté, Green & Hixon, 2013; Whitfield et al., 2002). They possess several venomous hard dorsal spines, pelvic spines, and anal spines, which act as deterrents and defence against many large-bodied predators (e.g. Groupers) (Halstead, Chitwood & Modglin, 1955).

The Lionfish species that have been identified to have invaded the Atlantic region are the Red Lionfish (*Pterois volitans*) and the Devil Firefish (*Pterois miles*). Within their native region, these Lionfish are almost geographically distinct (Whitfield et al., 2002), with few overlapping areas, and are morphologically distinguishable by their soft-ray fin counts (Morris, 2012; Akins et al., 2012). However, within the invaded regions of the Atlantic, though genetically distinct (Kochzius et al., 2003) these two species are morphologically identical (Côté, Green & Hixon, 2013; Morris & Akins, 2009; Morris, 2012).

Factors Facilitating the Spread of Alien-Invasive Lionfish

The impetus for their wide and invasive spread is most likely dispersal along oceanic current patterns dispersing the pelagic larvae along the Americas' coasts; successfully invading new locations. Lionfish rapidly expanded towards the east coast of the United States of America, Gulf of Mexico, and the Caribbean, and increased in abundance and population densities after their introduction (Côté, Green & Hixon, 2013; Green & Côté, 2009; Green et al., 2012; Morris et al., 2009, Schofield, 2009).

By 2012, Lionfish became an established "invader" in the Central and South Americas, the Caribbean Sea, and East of the Gulf of Mexico (Ruttenberg et al., 2012). Green and Cote (2009) estimated >390 Lionfish hectare-1 in the Bahamas; which was five times denser than in their native range.

Alien-Invasive Lionfish's Diet

Invasive Lionfish pose deleterious effects to an environment's overall health and functioning, almost exclusively due to their generalist diet and feeding strategies. Lionfish eat a wide variety of marine fishes and invertebrates (Côté, Green & Hixon, 2013; Morris & Akins, 2009; Muñoz, Currin & Whitfield, 2011). In their native range, they are opportunistic predators; consuming fishes, shrimps, and crabs (Muñoz, Currin & Whitfield, 2011; Ruiz-Carus, Matheson, Roberts & Whitfield, 2006). Côté, Green & Hixon (2013) reported that in the Bahamas, Lionfish prey included 57 species of reef fish from 25 families. Morris and Akins (2009) highlighted the 10 families (that included 41 species) that were most preyed upon by Lionfish within the Bahamian archipelago; Gobiidae, Labridae, Grammatidae, Serranidae, Pomocanthidae, Apogonidae, Monocanthidae, Atherinidae Mullidae, and Blenniidae. The study also noted that as Lionfish age, there's an ontogenetic shift from a crustacean-focused diet to a teleost-focused diet. This observation is most likely due to an increase in the gape-limit of their mouths (Côté, Green & Hixon, 2013). The generalist feeding nature of the Lionfish, lends to many trophic levels being affected, such as herbivores, piscivores, invertivores, omnivores, planktivores, detritivores, and cleaners (Eddy et al., 2016).

Lionfish habitats

Côté et al. (2013) describes Lionfish as ecological generalists, due to their tenacity to survive and thrive in many different types of ecosystems. Examples of the various ecosystems include temperate hard-bottom reefs (Whitfield et al., 2002, Whitfield et al., 2007), shallow mesotropic coral reefs (Albins & Hixon, 2008; Hixon et al., 2016), seagrass (Claydon et al., 2012), mangroves (Barbour et al., 2010), algal sand plains (Muñoz, Currin & Whitfield, 2011) and estuarine rivers (even in sections where it was almost entirely freshwater) (Jud et al., 2011). Lionfish seek to occupy any three-dimensional structure available, whether natural or man-made (e.g. shipwrecks, discarded fishing gear, artificial reefs, or other debris) (Smith, 2010; Côté, Green & Hixon, 2013; (Muñoz, Currin & Whitfield, 2011). They tend to seek shelter in dark, high-relief areas (Subramaniyan & Varadharajan, 2013).

Problems for the Fishing Industry

Caillet et al. (1986) classifies Lionfish as demersal meso-carnivores, like groupers and snappers. Due to prey naiveté and a lack of predators, these invaders (directly and indirectly) pose detrimental and long-lasting consequences to Atlantic reef fish populations, marine food webs, and furthermore the local and regional fishing industries. Like their native counterparts, the invasive Lionfish are generally crepuscular; however, they have been sighted actively feeding throughout the day and night (Côté and Maljković, 2010). This constant feeding pressure by such ravenous generalist predators can drastically reduce local fish stock and recruitment. It is estimated that an individual adult Lionfish (300–400g) in its native region, can consume ca. 8.5 g/day of prey (Fisherlon, 1997). However, due to ecological and environmental differences in the Atlantic region, invasive alien Lionfish may consume up to threefold that amount (Green, Akins & Côté, 2011), and estimations of their predation may be underestimated (Côté and Maljković, 2010). On a 30-minute survey, a single adult was observed to have consumed >20 small wrasses (*Halichoeres bivittatus*) (Albins and Hixon, 2008).

Lionfish Potential to Reduce Reef Fish Abundance and Recruitment

There have been several surveys to observe the overall reduction of reef fish populations and recruitment within locations invaded by Lionfish. Green et al. (2013) saw 65% reduction in 2 years, Albins and Hixon (2008) saw an 80% reduction in 5 weeks, and Albins (2013) saw 94% reduction in 8 weeks.

In its native range, Lionfish had few natural predators, and could attain sizes of 38 cm Total Length (TL) (Bernadsks and Coulet, 1991), and have recorded depths around 50 m (Nuttall et al., 2014, Randall et al 1990). However, in invaded territories, Lionfish can attain sizes ≥476 mm TL (Pusack et al., 2016; Morris, 2012), and exceed depths of 305 m (Muñoz, Currin & Whitfield, 2011).

Muñoz, Currin & Whitfield (2011) weighed the concern that invasive Lionfish pose a major threat to the biodiversity of any invaded region. They are also warned to have the potential to cause hundreds of billions of dollars in economic damage.

Alien-Invasive Lionfish's indirect influence on Eco-Tourism

Lionfish may cause deleterious changes to the coral reef functionality (Albins and Hixon, 2008). The fishing pressure produced by the Lionfish, compounded with other environmental factors ([over]fishing, pollution, loss of habitat, coral-algal shifts, and climate change) can deteriorate the quality of the ecosystems they invade. Such changes include the decrease of keystone herbivorous fishes (e.g., Parrotfishes, Wrasses, and Damselfishes), which may prompt an algal overgrowth in the area. Additionally, the reduction of overall fish populations and recruitment can reduce interest in water-based eco-tourism activities, such as snorkelling, SCUBA diving, and photography.

Alien-Invasive Lionfish's invasion into the Montego Bay Marine Park

The Montego Bay Marine Park (MBMP) is a monitored and protected area that also hosts two Special Fisheries Conservation Areas (SFCAs). Lionfish removal has been an intrinsic mandate for the Montego Bay Marine Park Trust (MBMPT) since the first semblance of the invasion in 2012 (authorized by then Executive Director Brian Zane, and current Executive Director Hugh Shim). The Trust actively cull Lionfish to protect their nurseries and fish stock. However, due to Lionfish's widespread and generalist tendencies, seeking and culling of the population was rigorous and laborious. Ultimately, the frequency, intensity, and yield of Lionfish culls became diminutive.

MBMPT Lionfish Removal Project

For this assessment, the Park was, as best as possible, systematically combed through; to visit as many distinct sites as possible. The expectation was that the information gleamed will help garner a larger and more accurate understanding of the local Lionfish population and distribution. It is understood that the Lionfish population within the Park may never truly be extirpated. However, if the population's general recruitment, aggregation, and spawning, zones can be discovered and delimited, then future culls can be more precise and have a greater impact on local populations.

The expected outcomes of this project are to conceive a comprehensive understanding of the population dynamics and the diet of the Lionfish within the Park.The information gathered from the specimens at each site would allow for a meticulous analysis of the composition (male to female ratio, sexual stages of maturity, and size classes) of not only a given site, but also the immediate surrounding areas. The data will aid in confirming the depths and habitats of Lionfish. Furthermore, gut content analysis would provide a more profound insight into what the Lionfish are preying upon within the Park. Length-weight graphs will also be generated to assist with any data analysis where only one of either parameters is known.

It is nigh impossible to exterminate the Lionfish from Atlantic waters through culling efforts alone. The extent to which they can inhabit far outreaches current conventional methods of population control (i.e., recreational SCUBA and free diving, FADs [Fish Aggregation Devices] and deep-sea netting and trawl). However, local populations can be targeted, to reduce impact and spread along the coastline of island states.

In Little Cayman, there were a series of Lionfish culls/rallies which specifically targeted invaded sites. The observation after 7 months was a reduction in the overall number and mean size of the Lionfish population in the areas (Frazer et al., 2012). Green et al. (unpublished) alluded to culling activities, even partial ones, can halt the erosion of biomass of native fishes.

The results from this research would be the impetus for more thoughtful and precise culling planning and activities. The initial expected outcome would be higher catches of Lionfish, eventually followed by a general decline in sightings and recruitment.

Methodology

Lionfish were collected from discrete locations (n = 41) within the MBMP during 2018. Sample sites were accessible sections of the Park (either by foot or by boat). The targeted locations for Lionfish removal included (high-, medium-, and low-profile) continuous and patch coral reefs, artificial reefs/structures, mangroves, seagrasses, marinas, and rocky shores, with depths ranging between 1 – >120 ft.

Confirmed Lionfish sightings were recorded year-round, whether seen on active culls, while engaged in other research/ maintenance activities, or through surface sightings (either by shore, snorkelling, or through a glass-bottom boat). Meta-Data recorded included date and time, site and location within the Park, over-head weather condition, depth range observed, and habitat type(s) observed. Observation sessions were scheduled to include periods of low light (dusk, dawn, throughout the night, and extremely overcast days). Therefore, time segments ranged from 00:00 to 23:59, with delimitations of the time of day.

Specimens were collected by MBMPT staffers, trained volunteers, and commercial fishers, while using snorkelling or SCUBA gear, Hawaiian slings or pole spears, hand-held nets, or standard fish traps. Where possible, Lionfish were placed in a transport container to minimize the loss of stomach regurgitation during ascension. Additionally, the numbers and total-lengths (in mm) of Lionfish seen but not captured (i.e., missed) were also estimated per session. In the event a Lionfish evaded capture, measuring tape was used to scale the spot the Lionfish was seen, to produce an accurate estimation of the TL.If possible, Lionfish were dissected the same day as capture (within 4 hours) or placed in a freezer (-15 °C) until the next opportune time. Prior to dissection, Lionfish were measured for Standard-Length (SL; in mm), Total-Length (TL; in mm), and Weight (W; in grams). The alimentary system of each Lionfish was dissected, and the gut contents were observed by eye. All remnants of prey items were identified to the lowest possible taxonomic level, enumerated, and measured for Total-Length (TL). Unidentified teleosts were grouped based on if the TL was less than 20mm, or greater than or equal to 20mm. Furthermore, teleost remnants that had no discernible form or feature, and underwent excessive digestion were grouped into "Indistinguishable Fish Matter"; likewise for excessively digested crustaceans which were grouped into "Indistinguishable Crustacean Matter".

The occurrence method was used to express the Lionfish's preference of prey items in percentages of total gut content collected. The following relative metrics of prey quantity: percent frequency of occurrence (%F) and percent composition by number (%N) were calculated to identify contribution of each prey taxon to the overall diet (Morris & Akins, 2009; Hyslop, 1980).

Results

Meta Data

As shown in Table 1, 117 Lionfish data collection sessions were conducted. During sampling, 1,126 individuals were seen, with 902 caught and dissected, (224 Lionfish evaded capture and/or were inaccessible at the time). Lionfish were observed to exist within depth ranges of 1–168 ft. and occupied seven (7) distinct habitat types. Lionfish were sighted within or around (low-, medium-, and high-profile) coral reefs, (low-profile) artificial reefs, Sand patches, Rocky/Rubble Patches, Seagrass Meadows (Table 2).

Lionfish Dissection Data

The Lionfish collected ranged in size from 65–394 mm TL (Table 3). Lionfish were arranged based on size classes (Table 4). Additionally, length-mass relationships and length-frequency information were plotted (Figures 1, 2, and 3).

The data plotted shows that as the length increases, the weight of the Lionfish increases exponentially. The largest Lionfish had a SL of 305 mm / TL of 394 mm, with a weight of 605 g, whilst the smallest Lionfish had a SL of 47 mm/ TL of 65mm, with a weight of 4 g.

Most of the specimens were within the total length size classes of 201-250 mm and 151–200 mm. No Lionfish captured exceeded 400 mm in total length.

The total numbers of captured females and males were 424 and 478 respectively.

Table 1: Summary Table of general statistics for Lionfish data collection sessions for 2018

Number of Lionfish Data Collection Sessions	117
Number of Early Morning Sessions (05:00–07:00)	5
Percentage of Total Sessions;	4
No. Lionfish accrued over time	53
Number of Morning Sessions (07:0–1200)	51
Percentage of Total Sessions	44%
No. Lionfish accrued over time	465
Number of Afternoon Sessions (12:00–17:00)	28
Percentage of Total Sessions	24%
No. Lionfish accrued over time	188
Number of Evening & Night Sessions (17:00–05:00)	33
Percentage of Total Sessions	28%
No. Lionfish accrued over time	420
Minimum Depth Seen/ Caught at (ft.)	1
Maximum Depth Seen/ Caught at (ft.)	168
Total Lionfish Seen	**1,126**
Total Lionfish Caught	**902**
Total Lionfish Missed	**224**

Table 2: Summary table of novel habitat types visited during Lionfish data collection sessions in 2018

Habitat Type	No. of Occurrences	Percentage of Occurrences
Low-Profile Coral Reef (Rel. Low Rugosity*)	28	13%
Medium-Profile Coral Reef (Rel. Medium Rugosity)	76	36%
High-Profile Coral Reef (Rel. High Rugosity)	59	28%
Low-Profile Artificial Reef (Rel. Low Rugosity)	2	1%
Medium-Profile Artificial Reef (Rel. High Rugosity)	0	0%
High-Profile Artificial Reef (Rel. High Rugosity)	0	0%
Sand Patch	26	12%
Rocky/ Rubble Patch	15	7%
Seagrass Patch	3	1%
Mangrove	0	0%
UKNOWN	5	2%
Total	**214**	**100%**

*Rugosity was based on presence and occurrence of Crevices, Overhangs, Ledges, Tunnels, and/or Grooves.

Table 3: Dissection Data Summary for Lionfish Collections

Smallest Standard Length (mm)	47
Largest Standard Length (mm)	305
Average Standard Length (mm)	156
Mode Standard Length (mm)	150
Smallest Total Length (mm)	65
Largest Total Length (mm)	394
Average Total Length (mm)	207
Mode Total Length (mm)	230
Lightest Lionfish (g)	4
Heaviest Lionfish (g)	615
Average Weight (g)	126
Total No. Females Caught	**424**
Total No. Males Caught	**478**
Total Lionfish Caught	**902**

Table 4: Tally of number of Lionfish within Total Length Size Classes

No. Lionfish ≤100 mm	10
No. Lionfish 101 - 150 mm	150
No. Lionfish 151 - 200 mm	263
No. Lionfish 201 - 250 mm	294
No. Lionfish 251 - 300 mm	147
No. Lionfish 301 - 350 mm	32
No. Lionfish 351 - 400 mm	6
No. Lionfish >400 mm	0
Total Lionfish	**902**

Females and males were also grouped based on sexual stages of development (Figure 4).

Most of the females were "Developing" and "Early Developing". The males were almost evenly "Mature" and "Immature".

Prey Data

In total, 1,270 prey items were removed from 649 stomachs and were measured and identified (Table 5). A total of 253 stomachs were found empty. Most prey items identified were ≤10 mm. Few prey items exceeded 50 mm.

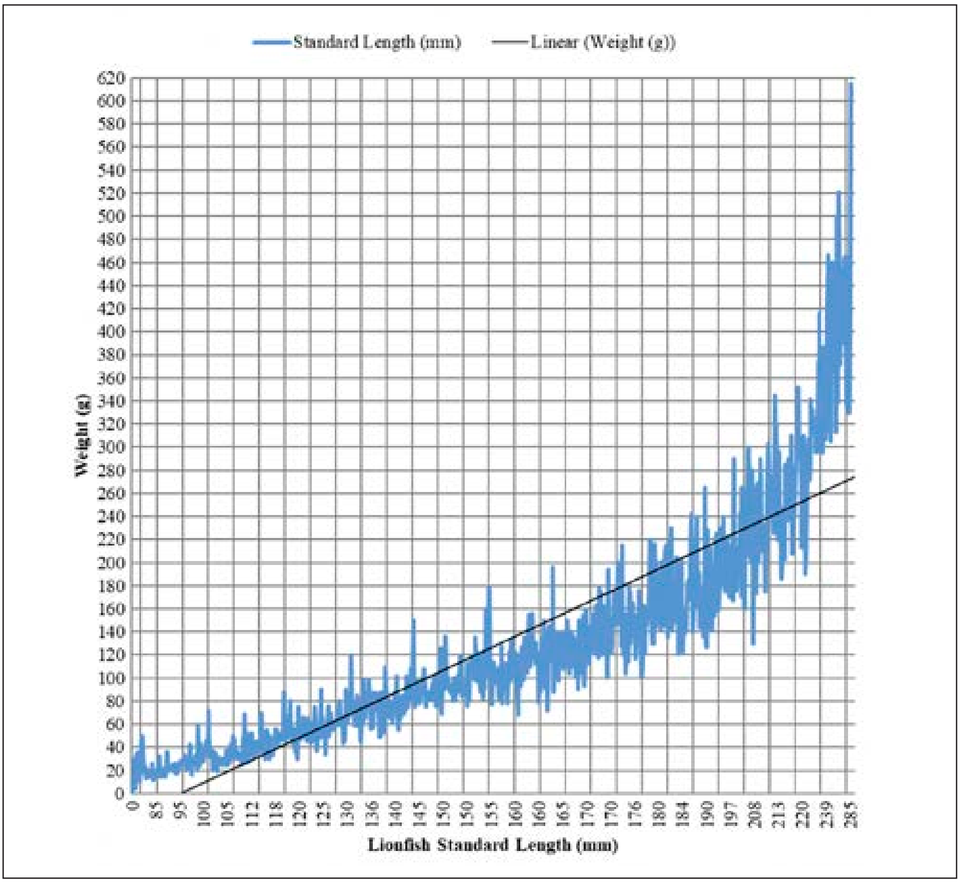

Figure 1: Lionfish (Pterois sp.) Standard Length (mm) vs Weight (g)

The five family/ categories with the longest prey TL lengths were Serranidae (80 mm), Scaridae (100 mm), Pomacanthidae (100 mm), Labridae (110 mm), and Indistinguishable Fish Matter (130 mm). Furthermore, the identifiable teleost classes with prey counts >10 tallies were Pomacanthidae (n=11), Scaridae (n=14), Grammatidae (n=14), and Holocentridae (n=15) (Table 6).In total, 13 families of teleosts, 3 families of crustaceans, and 1 family of molluscs were identified in the diet of the Lionfish.

Teleosts accounted for 56.5% of the total gut content by number (%N) (n = 718) and occurred in 61.1% of stomachs (%F). Crustaceans accounted for 43.3 % of the total gut content by number (%N) (n = 550) and occurred in 38.7% of stomachs (%F). Molluscs accounted for 0.2% of the total gut content by number (%N) (n = 2) and occurred in 0.2% of stomachs (%F).Teleost prey included 27 identifiable species. The families with the highest species richness were Scaridae (4 spp.), Labridae (4 spp.), Pomacanthidae (4 spp.), Holocentiridae (3 spp.), Lutjanidae

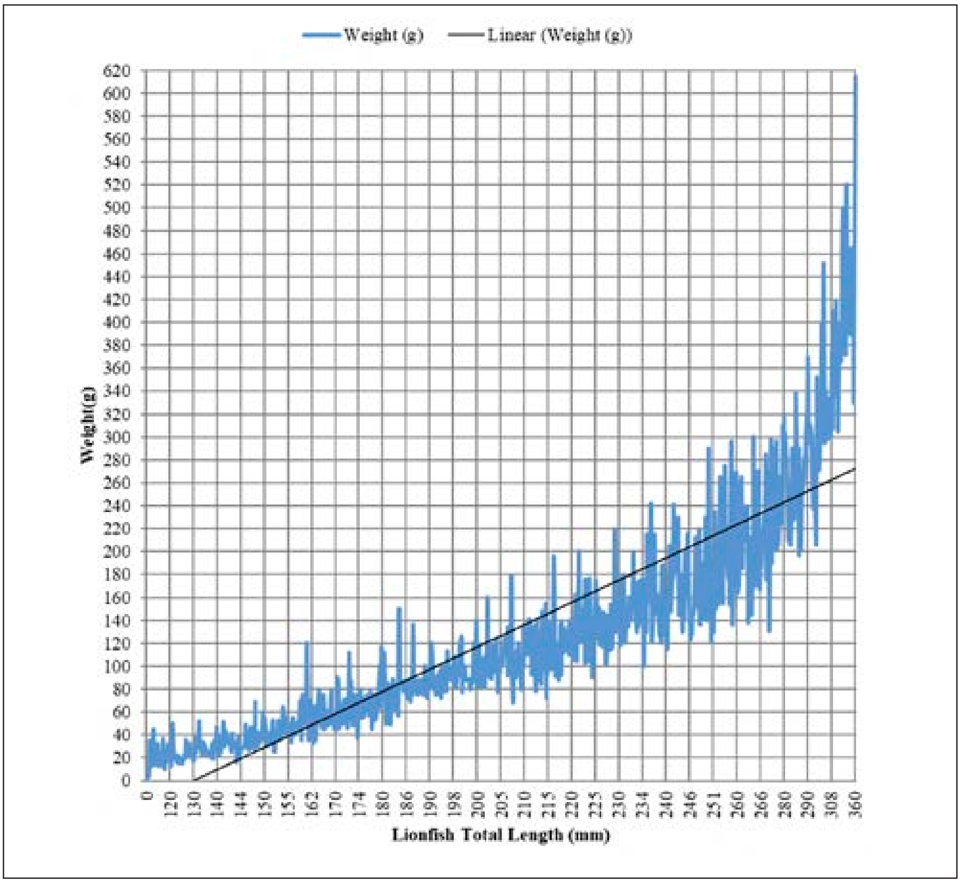

Figure 2: Lionfish (Pterois sp.) Total Length (mm) vs Weight (g)

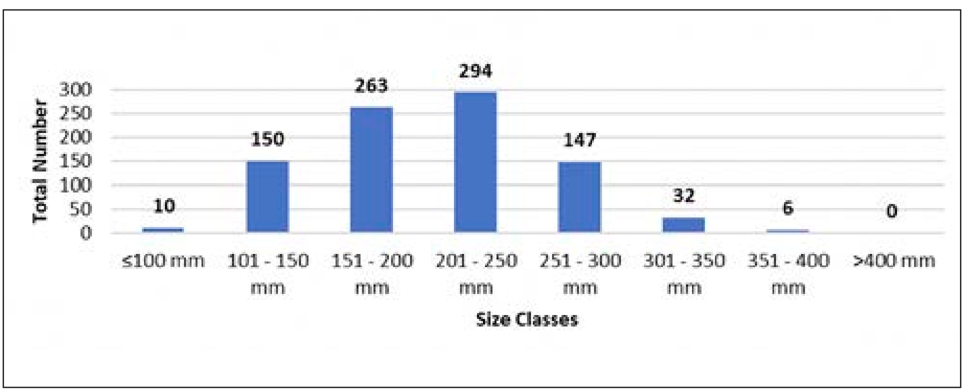

Figure 3: Captured Lionfish (*Pterois spp.*) arranged by length-frequency

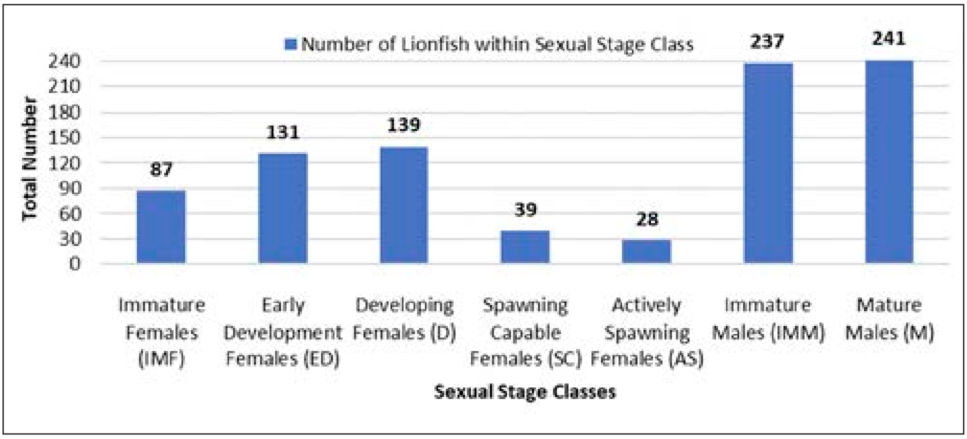

Figure 4: Captured Lionfish (*Pterois spp.*) arranged by sexual stage of development class tallies

Table 5: Tally of number of prey individuals/items per size class

Size Class	No. Prey Individuals	Size Class	No. Prey Individuals
≤10 mm	448	36 - 40 mm	31
11 - 15 mm	344	41 - 45 mm	10
16 - 20 mm	205	46 - 50 mm	22
21 - 25 mm	73	51 - 55 mm	5
26 - 30 mm	77	56 - 60 mm	5
31 - 35 mm	37	>60 mm	13
	Total 1270		

(2 spp.), Serranidae (2 spp.), and Haemulidae (2 spp.). These 7 teleost families made up 5% of the overall known diet by number (%N) and 5.9% of the overall occurrences (%F).

Crustacean prey included 3 identifiable species (Table 8). The families identified were Rhynchocinetidae (1 spp.), Pseudosquillidae (1 spp.), Xanthidae (1 spp.) The 3 crustacean families made up 26.8% of the overall diet by number (%N) and 15.8% of the overall occurrences (%F).

Mollusc prey included 1 identifiable species. The Loliginidae family had only 1 spp. and contributed 0.2%N and 0.1%F to the overall diet.

The categories of Unidentified Teleosts (<20mm and ≥20mm), Indistinguishable Fish Matter and Indistinguishable Crustacean Matter contributed the largest proportions of the Lionfish diet contributing 66.1%N and 75.9%F.

Table 6: Summary of prey item families and respective total counts and lengths

Family/ Category	Total Count	Min. TL (mm)	Max TL (mm)	Avg. TL (mm)	Mode TL (mm)
Teleosts	718	3	130	34.97	12
Scaridae	14	20	100	51.57	40
Labridae	8	12	110	44.50	#N/A
Holocentridae	15	23	68	38.80	40
Pricanthidae	1	35	35	35.00	#N/A
Lutjanidae	5	20	60	32.00	20
Aulostomidae	3	20	26	23.67	#N/A
Grammatidae	14	15	60	30.57	30
Acanuridae	3	30	30	30.00	30
Balitidae	4	20	36	27.75	20
Syngnathidae	1	20	20	20.00	#N/A
Pomacanthidae	11	30	100	52.00	50
Serranidae	6	35	80	49.17	35
Haemulidae	5	35	110	61.60	#N/A
Unidentified Fish (<20mm)	192	6	19	14.33	12
Unidentified Fish (≥20mm)	81	6	60	28.74	20
Indistinguishable Fish Matter	355	3	130	19.81	12
Crustaceans	550	1	40	15.84	7
Rhynchocinetidae	327	1	30	6.85	4
Pseudosquillidae	3	20	40	30.00	#N/A
Xanthidae	10	10	20	15.20	20
Indistinguishable Crustacean matter	210	2	40	11.30	12
Molluscs	2	23	24	23.50	#N/A
Loliginidae	1	24	24	24.00	#N/A
Unidentified Molluscs	1	23	23	23.00	#N/A
Empty	253	–	–	–	–
Total Prey Items Counts	**1270**	**–**	**–**	**–**	**–**

Table 7: Identifiable prey items sorted by taxa

		Occurrences in Stomachs	Number of prey items counted	Families % F (n = 918)	Families % N (n = 1270)
Teleosts species		560	718	61.2%	56.5%
Scaridae	Scarus inseri	3	5	1.2%	1.1%
	Sparisoma viride	1	1		
	Sparisoma aurofrenatum	2	2		
	Scaridae sp.	5	6		
Labridae	Thalassoma bifasciatum	2	2	0.9%	0.6%
	Clepticus parrae	1	1		
	Bodianus rufus	1	1		
	Labridae sp.	4	4		
Holocentridae	Holocentrus adscensions	6	7	1.4%	1.2%
	Sargocentron coruscum	1	1		
	Myripristis jacobus	6	7		
Pricanthidae	Pristigenus arenatus	1	1	0.1%	0.1%
Lutjanidae	Lutjanus apodus	1	1	0.3%	0.4%
	Lutjanidae sp.	2	4		
Aulostomidae	Aulostomus maculatus	3	3	0.3%	0.2%
Grammatidae	Gramma loreto	12	14	1.3%	1.1%
Acanuridae	Acanthurus sp.	2	3	0.2%	0.2%
Balitidae	Balitidae sp.	2	4	0.2%	0.3%
Syngnathidae	Hippocampus sp.	1	1	0.1%	0.1%
Pomacanthidae	Abudefduf saxatilis	1	1	1.1%	0.9%
	Stegastes adustus	7	8		
	Chromis cyanea	1	1		
	Microspathodon chrysurus	1	1		
Serranidae	Serranus tigrinus	3	3	0.5%	0.5%
	Serranidae sp.	2	3		
Haemulidae	Haemulon flavolineatum	2	2	0.5%	0.4%
	Haemulon sp.	3	3		
–	Unidentified Fish (<20mm)	83	192	9.1%	15.1%
–	Unidentified Fish (≥20mm)	47	81	5.1%	6.4%
–	Indistinguishable Fish Matter	355	355	38.7%	28.0%

Table continues

Table 7: Identifiable prey items sorted by taxa (*cont'd*)

		Occurrences in Stomachs	Number of prey items counted	Families %F (n = 918)	Families %N (n = 1270)
Crustacean Families		355	550	38.7%	43.3%
Rhynchocinetidae	Cinetorhynchus manningi	135	327	14.7%	25.7%
Pseudosquillidae	Pseudosquilla ciliata	3	3	0.3%	0.2%
Xanthidae	Xanthidae sp.	7	10	0.8%	0.8%
–	Indistinguishable Crustacean Matter	210	210	22.9%	16.5%
Mollusc Families		2	2	0.2%	0.2%
Loliginidae	Sepioteuthis sepioidea	1	1	0.1%	0.1%
–	Unidentified Molluscs	1	1	0.1%	0.1%
TOTAL		**918**	**1270**	**100.00%**	**100.00%**

Table 8: Summary of site descriptions and respective Lionfish statistics

Site Name	Zone in Park	General Depth Profile (ft.)	Habitat Class(es) Within Site	No. Sessions	No. Lionfish Seen	No. Lionfish Caught
Tropical Beach's Reef	NE	30 - >100	1, 3	1	17	10
(North of) Airport's Reef	NE	30 - >100	1, 2, 7	2	16	16
Airport's Reef	NE	12 - >70	1, 2, 3	2	12	12
Widow Maker	NW	50 - >100	2, 3, 7	1	18	16
Mason's Reef	NW	40 - >130	2, 3	12	89	75
(South of) Mason's Reef	NW	40 - >130	2, 3	1	5	3
Jack Tar Reef	NW	12 - 35	1, 2, 3, 7	3	5	4
Eco Reef	NW	26 - 55	1, 2, 4	1	1	0
Radical Cliff	NW	30 - >100	2, 3, 7	3	27	19
Total					**190**	**155**
M018	NC	20 - 45	1	1	1	1
APSFCA B2 Demarcation	NC	35 - >100	2, 3, 7	2	19	18
Little Paradise	NC	10 - >100	2, 3	2	10	9
Doctor's Cave Beach's Reef	NC	3 - >40	1, 7, 8	2	31	28

Table continues

Table 8: Summary of site descriptions and respective Lionfish statistics (*cont'd*)

Site Name	Zone in Park	General Depth Profile (ft.)	Habitat Class(es) Within Site	No. Sessions	No. Lionfish Seen	No. Lionfish Caught
Doctor's Cave's Beach	NC	3 - 15	1, 2, 7	12	75	46
Casa Blanca's Rock Wall	NC	2 - 15	7, 8	1	3	3
Cammock's Hammock	NC	12 - 45	1, 2, 7	4	52	41
May Way	NC	10 - 45	2, 7	2	13	8
The Office	NC	12 - 35	1, 2	2	20	16
Shim's Canopy	NC	25 - >130	2, 3, 7	10	105	76
Old Hospital Beach's Demarcation	NC	30 - 80	1, 2, 3, 8	6	82	60
Kramer's Cracks	NC	25 - >100	1, 2, 3, 8	2	35	24
Walter Fletcher Beach's Demarcation	NC	2 - 40	1, 2, 7, 8	1	16	13
Walter Fletcher Beach's Reef	NC	3 - >40	1, 2, 8	1	42	36
Total					**504**	**379**
Pier One's Dock	SC	1 - 4	8	3	19	8
Pier One's Rocks	SC	2 - 15	8	6	121	121
Yacht Club Channel Markers	SC	20 - 25	7	1	5	5
Yacht Club & Cruise Ship Port	SC	3 - 30	4, 7, 8	1	10	8
Inner Sea-Wind Peninsula	SC	6 - >45	1, 2	1	9	8
(NW of) Sea-Wind Peninsula to Sunscape Reef	SC	30 - 80	2, 3	1	2	2
Ben-Up Plateau	SC	30 - >100	2, 3, 7	2	20	14
Hard Rock Café's Reef	SC	30 - 90	2, 3, 7	3	28	19
Secrets' Reef	S	15 - 25	2	1	2	2
Chill Spot	S	20 - >100	2, 3, 7	5	66	52
Cammock's Lost World	S	40 - >100	2, 3	4	59	43
Bogue Lagoon Channel Markers	S	20	9	1	1	1
Total					**342**	**283**

Table continues

Table 8: Summary of site descriptions and respective Lionfish statistics (*cont'd*)

Site Name	Zone in Park	General Depth Profile (ft.)	Habitat Class(es) Within Site	No. Sessions	No. Lionfish Seen	No. Lionfish Caught
Secrets' Villas' Dock	SE	4	7, 9	1	27	27
Bogue Lagoon	SE	12	9	1	7	7
Classroom Reef (Heading East)	SW	40 - >140	2, 3	3	11	11
Spanish Anchor (Heading East)	SW	40 - >100	2, 3	1	3	0
Spanish Anchor (Heading West)	SW	30 - >100	2, 3	2	12	10
Total					**60**	**55**
Unknown (unrecorded and non-specific site selections throughout the Park)	Unknown	< 20 - >140	–	5	30	30

Location in Park Key:
NE: North-Eastern, NW: North-Western, NC: North-Central, SC: South-Central, S: Southern, SE: South-Eastern, SW: South-Western, Unknown: Unknown

Habitat Class Key:
1 - Natural Reef (Rel. Low Rugosity)
2 - Natural Reef (Rel. Med Rugosity)
3 - Natural Reef (Rel. High Rugosity)
4 - Artificial Reef (Rel. Low Rugosity)
5 - Artificial Reef (Rel. Med Rugosity)
6 - Artificial Reef (Rel. High Rugosity)
7 - Sand Patch
8 - Rocky/ Rubble
9 - Seagrass
10 - Mangrove
Unknown - Unknown

Site Data

Lionfish were sighted and/or removed from 41 discrete site locations within the Park; inclusive of an "Unknown Location" category. These sites ranged from the most northern sections to the most southern sections of the Park, as well as from near sea-level to sites deeper than 120ft.

Of the 41 sites, 9 were from the North-Western/ Eastern (NW/ NE) sections, 14 from the North Central (NC) section, 12 from the South-Central (SC) section, 5 from the South/ South-Western/ Eastern (S/SW/SE) sections, and the unknown locations were pooled into an "Unknown" category.

Each site was analysed for the general location in the Park, depth profile, habitat class(es) within the site, number of Lionfish seen and caught, lengths of Lionfish seen/ caught, and sex and maturity of Lionfish caught (Table 8). The sites within each general zone of the Park were grouped based on their geo-spatial proximity as well as their commonality in sub-marine topographies.

Conclusion

This research has provided the first site-focused Lionfish sampling within the MBMP, and will be used to validate and coordinate future lionfish management strategies within and around the Montego Bay area. The trends seen at "Pier One Rocks" and "Shim's Canopy" lend credence to the notion that sustained culling within a localized area can significantly reduce lionfish population over time. Having focused culls at the identified sites with proportionate adolescent and adult populations will optimistically reduce the overall population within the Park.

Lionfish predation on members of Scaridae, Larbridae, and Pomacanthidae families may play a role in indirectly facilitating the coral-algal phase shift along sections of coral reefs within the Park. Further research topics could be derived from the results gathered in this report.

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Journal of Arts, Science and Technology

Emerging Trends in Data Utilising Longitudinal Analysis

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Abstract

The aim of the study was to analyse trends in data utilising longitudinal data analysis. The focus was on two data applications which are (i) "Divorce rates versus success in school" and (ii) "Sustainable Development Goals (SDG) versus incidence of multidrug resistant tuberculosis (MDR-TB)". There were various methods that were used to analyse the two datasets. For the data application: "What are the effects of parental divorce on students' academic performance?" Linear mixed model (LMM) was utilized. For the data application: "Do SDG indicators influence the incidence of multidrug resistant tuberculosis?", data analysis was carried out using fixed and random effects models. There were four final models that were fitted in an effort to answer the hypothesis: "what are the effects of parental divorce on students' academic performance?" The two models involving reading ($p=0.035$) and history ($p=0.0271$) scores indicated that marital status impacted these scores significantly while the math and science scores did not have a significant statistical association with marital status. For the data application: "Do SDG indicators influence the incidence of MDR-TB?", the full random effects model indicated that the percentage of the population living below the international poverty line ($\beta=254.276$, $p=8x10^{-6}$) and the percentage of the population suffering from undernourishment ($p=0.032$) were statistically significant in their association with the incidence of MDR-TB.Increased divorce rates have a negative effect on academic success and Sustainable Development goal indicators influence the occurrence of multidrug resistant tuberculosis cases.

Keywords: Longitudinal, Linear Mixed Model, Random Effects Model and Fixed Effects

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Introduction

In this paper, the authors are exploring emerging trends in data utilising longitudinal analysis. Longitudinal studies are typically observational studies wherein a participant's outcomes, and possible treatments or exposures, are collected at multiple times on the same individual. These studies have no limit on the timespan and as such, can last up to several decades. Though shorter time frames are not recommended, the key is for the study to extend beyond a singular time point, ideally three or more (CLOSER, 2020). Conducting longitudinal studies is beneficial as it allows one to follow subjects in real time, which allows researchers to have a better understanding of the sequence of events (CLOSER, 2020). Furthermore, they provide the researcher with a clear timeline of how they reached a specific conclusion.

The longitudinal analysis model explored in this study is the Linear Mixed Model (LMM) among other models. In this paper, the focus will be on two data applications. The data applications are: "divorce rates versus success in school" and "Sustainable Development Goals (SDG) versus incidence of multidrug resistant tuberculosis (MDR-TB)". The research question for data application#1 is: *What are the Effects of Parental Divorce on Students' Academic Performance?* Students' academic performance is affected by so many things and parental divorce may be one of the key factors in this. This study adds to the body of knowledge concerning factors that affect academic performance which is a vital building block for youths. Students were surveyed and their experiences at school/work/home, neighborhood characteristics, and family structure were explored.

The research question for data application#2 is: *What are the Social Development Issues that are most Associated with Multidrug-Resistant Tuberculosis (MDR-TB) in 18 High Burden Tuberculosis Countries?* Access to drugs to treat the initial onset of tuberculosis poses a great challenge to the overall treatment of the disease. Improper treatment is most likely to lead to relapse cases of tuberculosis and multidrug resistant tuberculosis is more likely to be among these relapsed cases. Sustainable Development Goals (SDG) indicators are taken into consideration and these paint a picture of which countries are more likely to experience multidrug resistant tuberculosis. These are health expenditure per capita, percentage of population suffering from undernourishment, percentage of population living below the international poverty line, Gini Index, Gross Domestic Product (GDP) per capita and Percentage of urban population living in slums.

Health expenditure per capita tells the amount that each country spends on health, for both individual and collective services. Tuberculosis tends to affect poorer countries where access to medication, public information and the presence of full TB treatment plans may be limited. The risk of tuberculosis is exacerbated in undernourished populations. In patients suffering from tuberculosis, undernourishment leads to reduced appetite, nutrient malabsorption, micronutrient malabsorption, and altered metabolism, which leads to wasting. Undernourished tuberculosis patients have delayed recovery and higher mortality rates than well-nourished patients. This study seeks to explore how the SDG indicators affect the incidence of MDR-TB.

Methodology

There were various methods that were used to analyse the two datasets. For the data application: "What are the effects of parental divorce on students' academic performance?" Linear mixed model (LMM) and an unconditional means model were utilized. In this paper LMM will be expounded on. LMM is used to display continuous outcome measures as a function of fixed effects while modelling individual subject parameters as random effects. This model also assumes that the error term is structured according to a known hierarchy and thus may be called a multilevel model.

LMMs are probably one of the most widely used techniques for any form of longitudinal data analysis. The LMM, which contains both fixed and random effects is given by (West, Welch Gałecki & Gillespie 2015):

$$Y_i = x_i\beta + z_i d_i + e_i \quad \text{(Equation 1)}$$

Where, $y_i - n_i x\ 1$ vector of n observations on the ith subject

$\beta - p\ x\ 1\ vector\ of\ unknow, fixed\ population\ parameter$

x_i- n_i x p known, constant design matrix for the ith subject

$d_i -$ q x 1 vector of unknown, random individual parameters

z_i -n_i x q known, constant design matrix for the ith subject corresponding to the random effects d_i

e_i-n_i x 1 vector of random effect terms

For the data application: “Do SDG indicators influence the incidence of multi-drug resistant tuberculosis?”, data analysis was carried out using fixed and random effects models. Fixed effects models are statistical models in which the independent variables are fixed and the only dependent variable changes in response to the levels of independent variables (Zhang n.d.). Additionally, this model focuses on removing omitted variable bias by measuring the changes within groups across time by incorporating dummy variables for the omitted characteristics (Zhang n.d.). In longitudinal analysis, the fixed effects model represents the individual-specific means. A major assumption of this model, often called the fixed effect assumption, is that the individual specific effect is correlated with the independent variables. In essence, the fixed effects approach calculates the other coefficients in the model by holding the group fixed.

The equation for the fixed effects model is defined as:

$$Y_{it} = \beta_1 X_{it} + \alpha_i + e_{it} \quad \textit{(Equation 2)}$$

where Y_{it} is the outcome variable for the i^{th} subject at time t, α_i represents the unknown intercept for each entity, X_{it} represents the independent variable for the i^{th} individual at the t^{th} time, β_1 represents the coefficient for X_{it}, and e_{it} is the error term. Additionally, the fixed effects model can be visualized by using binary (dummy) variables, where the equation now becomes:

$$Y_{it} = \beta_o + \beta_1 X_{1,it} + \ldots + \beta_k X_{k,it} + y_2 E_2 + \ldots + y_n E_n + e_{it} \quad \textit{(Equation 3)}$$

where $E_2, \ldots, E_n$ are dummy variables. Equations 2 and 3 are equivalent since β on X is the same from one variable to the next, the variable specific intercepts in Equation 2 and the binary regressors in Equation 3 have the same source (Rodrıguez, 2012).

Data Collection and Information on Data Application #1

This dataset is taken from the Institute of Education Sciences (IES) database, the statistics arm of the US Department of Education. The dataset focuses on the National Education Longitudinal Study (NELS) of 1988, specifically from 1988-1992. A brief overview of this dataset would show that there are 6000+ variables and 26000+ observations on these variables, across three instances in time.

This study follows 8th grade students and records a myriad of information using a questionnaire, which has a biennial follow up. Some of the things the students are surveyed on include their experiences at school/work/home, neighborhood characteristics, and family structure. Students were also asked to self-report on after school activities and if they participate in drinking and/or smoking. Throughout the study, students were assessed in reading, mathematics, science and social studies at the 8th, 10th, and 12th grades. This study not only focused on students, but the teachers, parents and school administrators were also included in this study.

Data Collection and Information on Data Application#2

The data used in this study is taken from the World Health Organization (WHO). It focuses on panel/ longitudinal data taken from 18 different countries in 6 regions between 2009 and 2019. The countries involved are 3 countries with a high incidence of tuberculosis cases in their respective regions as seen in Table 1.

Table 1: Countries included in dataset and their respective WHO regions.

WHO Region	Countries
Africa (AFR)	Democratic Republic of the Congo Nigeria South Africa
Americas (AMR)	Brazil Mexico Peru
Europe (EUR)	Russian Federation Ukraine Kazakhstan
Eastern Mediterranean Region (EMR)	Afghanistan Pakistan Morocco
South East Asia (SEA)	Bangladesh India Indonesia
Western Pacific Region (WPR)	China Philippines Vietnam

The data were analysed using the R software.

Results

Data Application#1

Figure 1 shows the spaghetti plots created for the Math and Science standardized test scores. It shows the students' math and science scores increased over time as the blue line represents the average slope line. The black lines represent the individual trajectories.

Given that there was no single variable to assess students' academic performance, the subject based standardized scores were used. As such, there are four

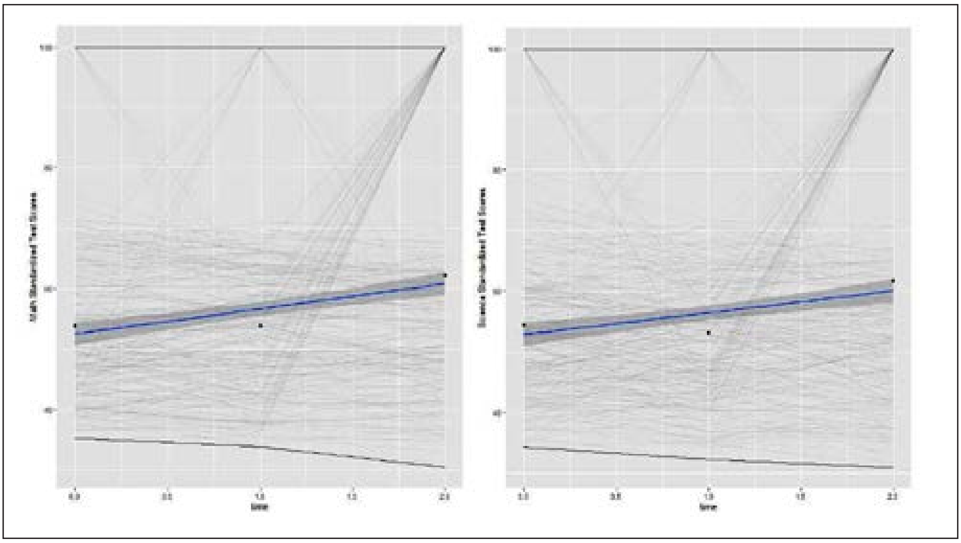

Figure 1: Math and Science standardized score of the students over time.

final models (see Table 2) that were fitted in an effort to answer the hypothesis: what are the effects of parental divorce on students' academic performance? The Reading model has a p-value of 0.035 for the marital status variable, resulting in a rejection of the null hypothesis stating that all the levels are the same. That is, we can conclude that a change in marital status significantly affects the students reading standardized score. In the Math model, we see that the p-value = 0.059 for the marital status variable, therefore we fail to reject the null hypothesis stating that all the levels are the same. That is, we can conclude that there is not enough evidence at $\alpha=0.05$, that a change in marital status significantly affects the students' mathematics standardized score. Looking at the science model, we note that the p-value for the marital status variable = 0.1233 which is clearly greater than $\alpha=0.05$, therefore we fail to reject the null. Additionally, looking at the history/CIT/geography model shows that marital status p-value = 0.0271 $< \alpha=0.05$, therefore we reject the null hypothesis and conclude that marital status affects the students' history/ CIT/ geography score.

Table 2: Linear mixed-effects model for the standardized scores and the independent factors for the four models (estimates shown and standard error in brackets).

	Reading	Math	Science	History/CIT/ Geography
Intercept	55.333 (0.559)	55.644 (0.555)	55.207 (0.563)	55.251 (0.564)
Parent's Marital Status	0.015* (0.007)	0.013 (0.0007)	0.011 (0.007)	0.016* (0.007)
Divorced @ 1st follow-up	1.331 (0.178)	1.325 (0.177)	1.372 (0.179)	1.428 (0.179)
Divorced @ 2nd follow-up	1.337 (0.193)	1.377 (0.192)	1.533 (0.195)	1.557 (0.195)

*Statistical significance at $\alpha = 0.05$

Data Application#2

Here we can see that for the incidence and number of deaths for tuberculosis, India has the greatest number of cases. As it pertains to TB incidence, China has the second largest incidence with over 1 million cases in 2009. Indonesia has the third largest incidence over time with a little less than 1 million cases in 2009. The

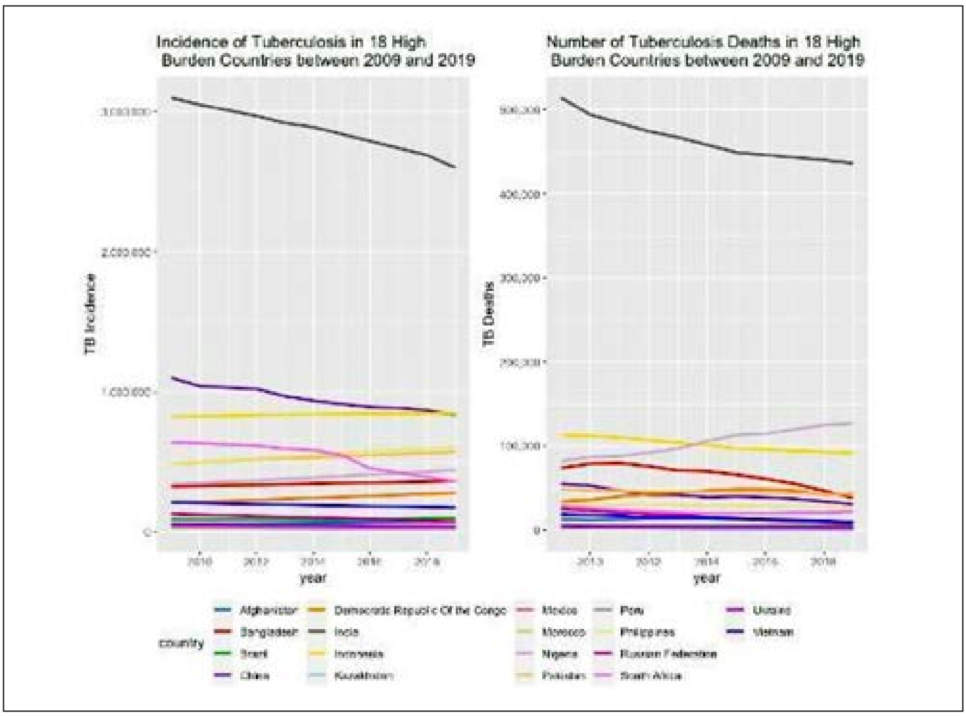

Figure 2: Graph showing the number of TB incidents and deaths by country.

incidence for China and Indonesia met in 2019. In general, there is a downward trend in the number of TB cases over time. For the number of tuberculosis deaths overtime, we can see that Nigeria has been experiencing an increase. It had the second highest number of TB deaths by the end of 2019.

The full random effects model indicated that the percentage of the population living below the international poverty line (β=254.276, p=8x10^{-6}) and the percentage of the population suffering from undernourishment (p=0.032) were statistically significant in their association with the incidence of MDR-TB (see Table 3). For every one percent increase in the percentage of the population living below the international poverty line, the incidence of the number of confirmed multidrug resistant tuberculosis cases increases by 254 persons. This is in line with the general notion that tuberculosis is a disease that is closely related to poverty.

Countries with higher percentages of undernourishment are more likely to have tuberculosis cases. From the dataset, for every one percent increase in the percentage of the population suffering from undernourishment, there is a 496 decrease in the number of persons affected by drug resistant tuberculosis. This

Table 3: Random Effects Model of the Number of Confirmed MDR-TB cases vs. all SDG Variables.

	Estimate	Std. Error	z-value	P-value	R-square	Adjusted R-square
Intercept	12190.280	8207.640	1.485	0.137	0.84601	0.76202
GINI	-187.120	183.776	-1.018	0.309		
% below international poverty line	254.276	57.195	4.446 -0.225	0.000008757		
GDP per capita	-0.082	0.366	-0.225	0.822		
% population undernourished	-496.708	232.206	-2.139	0.032		
% living in urban slums	0.052	0.731	0.070	0.944		
Health expenditure per capita	2.328	1.639	1.421	0.155		

result is not as expected and could be attributed to correlation among the independent variables in the model. The expected results would have been a positive sign for the undernourishment variable.

Conclusion

Increased divorce rates have a negative effect on academic success in reading and history/CIT/ geography subjects. Sustainable development goal indicators influence the occurrence of multidrug resistant tuberculosis cases.

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Simulation Driven Investigation of the Effect of SnS, ZnO and Mo(S,Se)$_2$ Layers on a GaAs/AlGaAs Heterojunction Solar Cell

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Abstract

The performance of three layers (SnS, ZnO and Mo(S,Se)$_2$) on a GaAs/AlGaAs wafer were simulated using COMSOL Multiphysics which investigated its current-voltage, current-wavelength and electric field penetration capabilities. This was done to compare which material is best suited to enhance the performance of the GaAs/AlGaAs heterojunction. It was found that the relative permittivity of the layer was the most influential parameter in determining the outcome of the cell with the layer having the highest permittivity (ZnO) allowing the greatest electric field penetration and therefore the most current across the voltage and wavelength sweeps.

Keywords: Solar Cell, Heterojunction, COMSOL, Relative Permittivity, Current, Voltage and Wavelength

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Introduction

Solar energy has become increasingly popular for providing sustainable, carbon free energy for commercial use. While this might be true, there are several key aspects that must be considered in order for the use of solar energy to be utilised at its fullest potential which begins by addressing the solar cells themselves.

The global solar cell market consists predominantly of silicon based thin-film, monocrystalline and polycrystalline solar cells which correspond to around 95% of the modules sold (Woodhouse, 2016). Such a lack in diversity may lead to market vulnerability if there are any supply chain disruptions. For example, if there is a computer chip shortage, which many modern solar cell systems depend on, then the availability of solar cells will no longer be able to meet the growing demand. As a result, such a potential hazard to solar energy development must be addressed if it is to be effectively used as a viable and sustainable energy source.

Additionally, the power conversion efficiency of commercially available solar cells averages at 20% (Zito, 2022). This relatively low efficiency is caused by the combined effect of the isolated cell's efficiency as well as the total system's efficiency. With this in mind, in order for more widespread use of solar cells, it is imperative that its efficiency be improved upon.

To compound this, the way in which solar cells are researched can also be regarded as antiquated in some respects. It is costly in both time and expenses to test novel materials for solar cells. Material scientists along with physicists must come up with and synthesise each new layer for a solar cell in varying concentrations and then test them individually over and over to study its effect. This process is then repeated for other materials slowly adding to the pool of knowledge with respect to which materials are best suited for improving the efficiency and overall performance of a solar cell. Therefore, in order to achieve optimal energy sustainability and efficiency, a novel approach to solar cell research and development must be adopted.

In addition to conventional silicon wafers, heterojunctions solar cells have gained much attention in recent years. It combines wafer based solar technology with thin-film technology thereby increasing its overall efficiency (AE Solar, 2022). Heterojunctions are able to achieve this by using three layers of light absorbing materials consisting of the thin-film layers are well as the conventional layers. Heterojunction cells also have a reduced surface recombination and better performance in warmer climates.

There are many parameters that affect the efficiency of a solar cell with the two main ones being the path length of the charge carriers and the recombination lifetimes. If the physical length of the cell is larger than the path length of the charge carriers, then they would recombine before meaningful work can be extracted from the device. Similarly, if the recombination lifetime is too short, then the charge carriers will recombine before a current can be developed. Of course, there are other parameters such as the metal contacts, relative permittivity and carrier mobility that also affect the cell's performance. Consequently, in order to optimise the performance of the solar cell, parameters must be appropriately chosen. However, this can prove difficult to ascertain which is why a novel approach must be assumed.

Simulation and modelling driven research have also become increasingly popular in recent years. Although it has not been very popular in the research and development of solar cells, there are some that are using simulations for improving solar cells. For example, 3-D simulation using COMSOL Multiphysics of a heterojunction was used to demonstrate that the addition of a $Mo(S,Se)_2$ layer increased the open circuit voltage from 0.46 to 0.513V (Zandi, 2020).

As a result, this study demonstrates the efficacy of 3-D simulation for the development and research of solar cells. It also investigates the effect of three layers (SnS, ZnO and $Mo(S,Se)_2$) of a $GaAs/Al_{0.25}Ga_{0.75}As$ heterojunction using the simulation software COMSOL Multiphysics. In particular, the electric field penetration and the current voltage curves will be studied in order to ascertain which material is best suited for improving the performance of the heterojunction.

Methodology

The geometry of the heterojunction comprised of three layers as shown in Figure 1. The top layer consisted of a length of 10μm, width of 4μm and height of 0.25μm while the middle and bottom layers both had a length, width and height of 10μ, 4μ, 1μ respectively.

The specific material parameters (electrical properties) were then added to each layer. Initially, the top layer was set to have the properties of tin sulphide (SnS), then Molybdenum compound ($Mo(S,Se)_2$) and then zinc oxide (ZnO) while the middle layer was set to have the properties of gallium arsenide (GaAs) and the bottom layer was set to have the properties of aluminium gallium arsenide (AlGaAs). The properties that were used are shown in Table 1.

Once the material parameters were set, the addition of the physics can be done. This entailed adding semiconductor and optical physics. For the semiconductor physics the doping concentrations were added with three layers of doping: two analytical doping and one geometric doping model. This approach is recommended by COMSOL when working on a 3D model. As such, the doping for each layer was added by first utilising a background analytical doping model across the entire layer that was set constantly at 1×10^{14} cm^{-3}, then another analytical doping area of uniform doping was set at the doping concentration unique to the material,

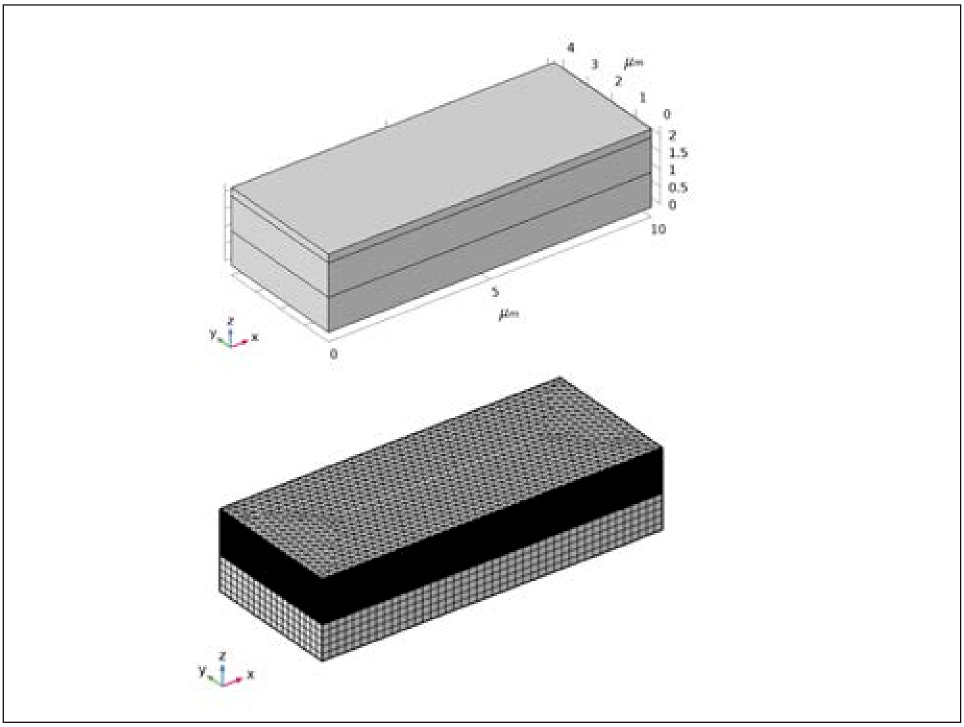

Figure 1: Geometry, dimensions, and mesh of the heterojunction wafer

Table 1: Electrical parameter used in the COMSOL Simulation

Parameter	SnS	$Mo(S,Se)_2$	ZnO	GaAs	AlGaAs
E_g (V)	1.64	1.10	1.10	1.42	1.74
χ (V)	3.36	4.14	4.40	4.06	3.80
ε_r	40	10	9	13.1	12.4
N_c (m^{-3})	3.6×10^{18}	0.7×10^{18}	2.2×10^{18}	4.36×10^{23}	6.55×10^{23}
N_v (m^{-3})	1.4×10^{19}	0.3×10^{19}	1.8×10^{19}	8.35×10^{24}	1.05×10^{25}
μ_n ($cm^2/(V\cdot s)$)	1	100	100	7000	2500
μ_p ($cm^2/(V\cdot s)$)	1	25	25	300	150
τ_n (ns)	0.014	2.4	0.003	10	10
τ_p (ns)	0.014	2.4	0.003	10	10
Doping (cm^{-3})	2×10^{12}	1×10^{15}	2×10^{12}	1×10^{15}	1×10^{16}

and finally a geometric doping layer was added such that a doping of what was specified to the layer was applied with a Gaussian decay which begins at 0.15μm away from the junction.

For the type of doping, the top layer was set with acceptor doping (p-type). The GaAs (middle) layer was set with acceptor doping (p-type) while the AlGaAs (bottom) layer was set with donor doping (n-type).

Additionally, the metal contacts were added such that the n- contact was at the top of the wafer while the p-contact was set at the bottom of the wafer.

Next the optical physics was added to the model by including the direction and magnitude of the incident electromagnetic radiation on the wafer as shown in Figure 2. The power of the radiation was set at 10μW and had normal incidence to the surface of the wafer.

Periodic boundary conditions were also added to the sides of the wafer such that the tangential components of the solution variables are equal on the source and destination. Finally, for a control experiment, the simulation was run with just the GaAs/AlGaAs layers to be used to compare the performance of the wafer with and without the added top layers.

A swept mesh was chosen for the geometry since this is the mesh that most accurately computes semiconductor physics in 3 dimensions as shown in Figure 1. The size of the mesh was set to 'finer'. A linked triangular mesh was applied to the top and bottom of the mesh. A swept mesh was then added to the vertical walls of the wafer. A uniform quadrilateral mesh was used for the top and bottom mesh with 20 and 5 elements respectively. An exponentially-distributed mesh with 200 elements was selected for the middle mesh. The quality of the mesh used was tested and is shown in Figure 6. As can be seen the mesh's quality was good since the mesh's quality was close to 1.

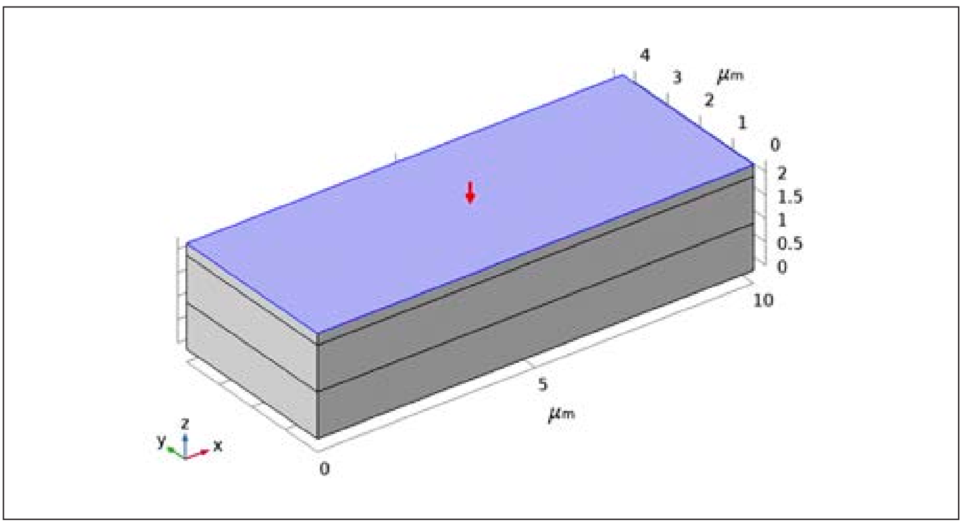

Figure 2: Incident electromagnetic radiation on wafer.

The wafers were tested using two criteria. For the first, a voltage sweep from 1V to 2V was applied. Secondly, a wavelength sweep from 475nm to 875nm was performed.

Results and Discussion

A current-voltage graph was produced that compared the performance of each of the wafers as shown in Figure 3. From the graph, it can be seen that the ZnO layer performed the best meaning that after 2V the current was the most. The ZnO layer had the lowest relative permittivity value which meant that it was the least polarised layer when exposed to the incident radiation and therefore was allowed to penetrate the wafer the most. This in turn allowed for greater electron-hole generation and therefore greater current production.

The $Mo(S,Se)_2$ and SnS layers did not perform well. This could be due to the formation of a Schottky barrier which is a potential energy barrier for electrons formed at a metal-semiconductor junction. Therefore, the charge carrier capability of the wafer could have been impeded enough to cause the low performance of the wafers.

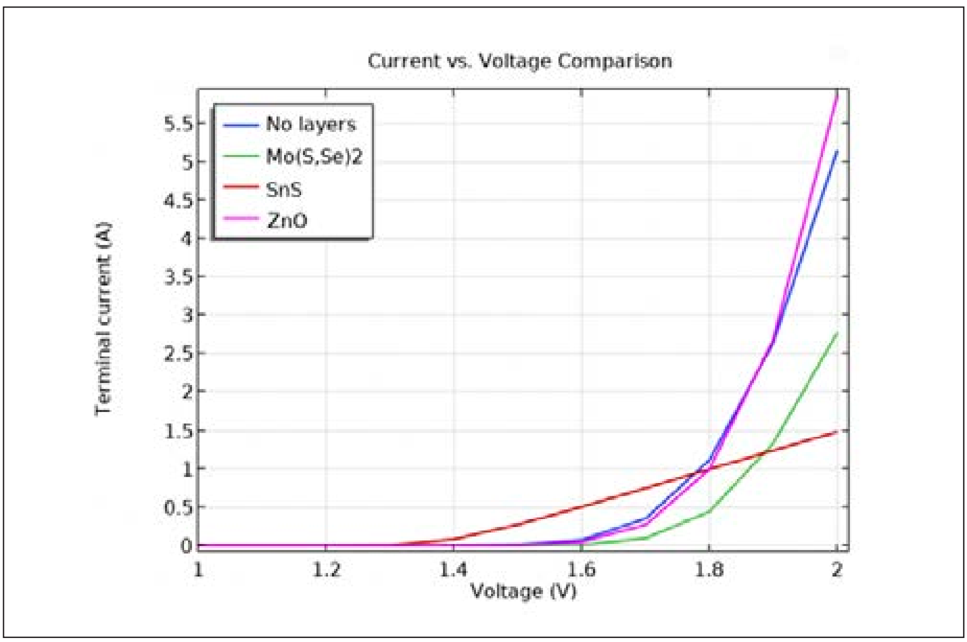

Figure 3: Current-voltage graph comparison

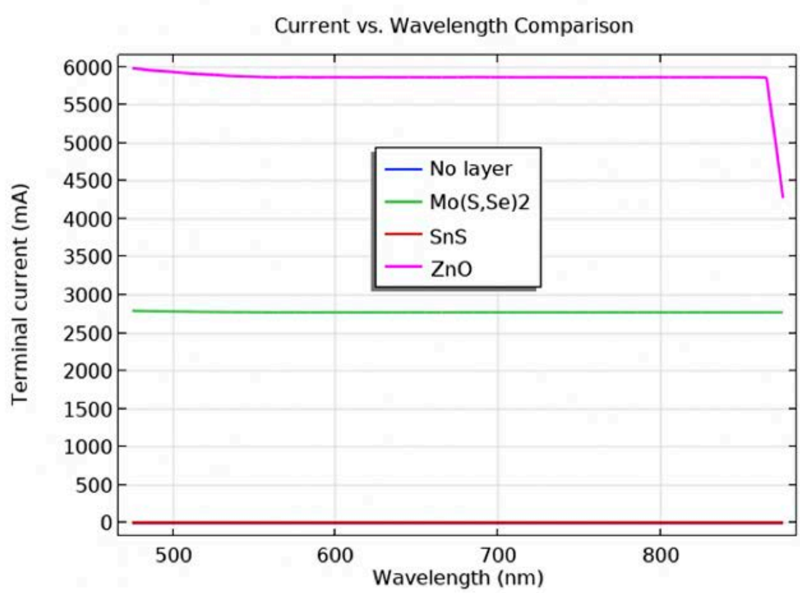

Figure 4: Current-Wavelength graph comparison. Note that the blue line is overlaid by the red line

The performance of the ZnO layer is further elucidated when the current-wavelength graph is studied as shown in Figure 4.

From Figure 4 it can be seen that the ZnO layer performed the best when it came to current production over the sweep of wavelength. This could be due to the fact that the relative permittivity value is the lowest thereby allowing the most electromagnetic radiation penetration. This is further compounded when looking at the performance of the SnS layer which had almost no current production over the wavelength range especially considering the fact that it had a relative permittivity of more than 4 times that of the ZnO layer. With this in mind, the Mo layer performed as expected since its relative permittivity was between that of the SnS and ZnO layers.

Further to this, Figure 5 shows the electric field penetration of the electromagnetic wave incident on the wafer. From the graph it can be seen that the ZnO layer allowed the most radiation through while the SnS layer did not. This is in keeping with the fact that the relative permittivity value was greater for SnS than it was for the ZnO layer. As a result it can be concluded that the relative permittivity played a significant role in allowing the ZnO layer to outperform the Mo and Sn layers.

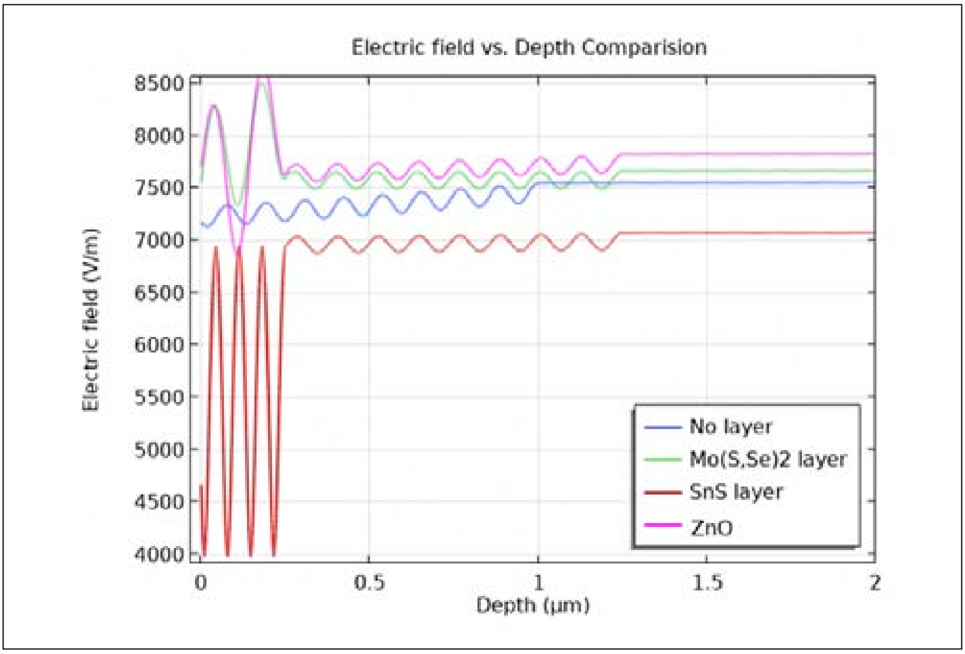

Figure 5: Electric field penetration based on the penetration of the electromagnetic radiation

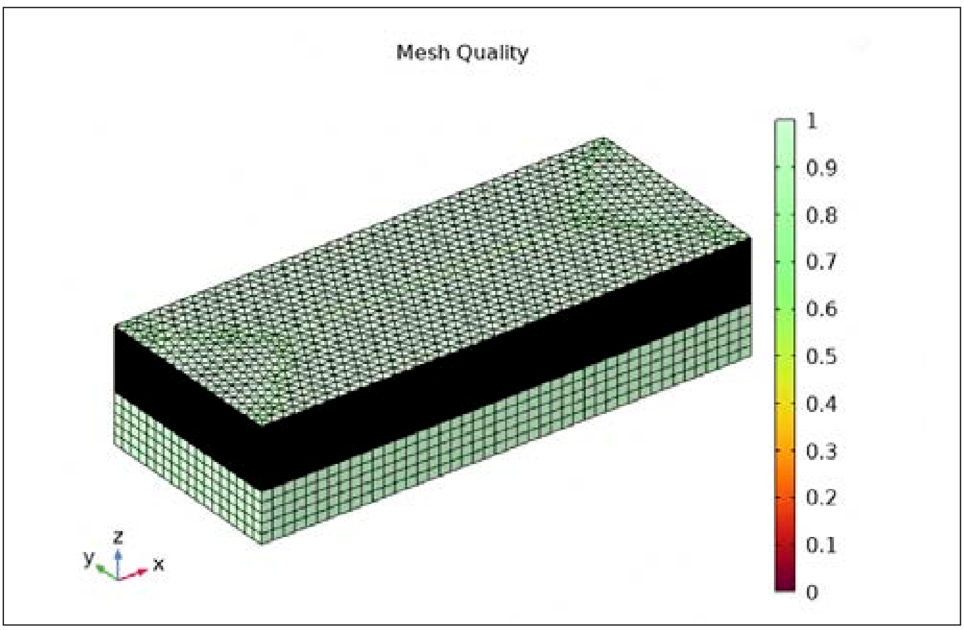

Figure 6: Quality of the mesh used

Conclusion

The wafer with the ZnO layer performed the best out of the three mostly because it had the lowest relative permittivity. As a result, when looking at the electric field penetration graph it had the highest penetration. Consequently, it produced the highest current values when swept over the wavelength range which lead to it producing the most current in the current-voltage graph. Conversely, the wafer with the SnS layer performed the worst because it had the highest relative permittivity being more than 4 times higher than that of ZnO. The Schottky barrier could also be the cause for the low performance of the MnO and SnS layers since it underperformed the wafer without any layers at all. Finally, based on the performance of the layers, depending on the value of the relative permittivity, the effects of the Schottky barrier can be overcome as was demonstrated by the ZnO layer.

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Evaluation of the Inter-Annual Variability of Site-Corrected Daily Global Horizontal Solar Irradiation in Trinidad and Tobago

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Abstract

The bankability of a solar photovoltaic system is dependent on the variability of the long-term solar resource and the accuracy of the solar radiation data considered. The evaluation of the accuracy and variability of the long-term solar resource is of high importance to Trinidad and Tobago as the nation is moving towards installing their first grid-scale photovoltaic systems. Project developers need reliable long term solar resource data which reflect site-specific trends and inter-annual variability of the solar resource. In evaluating the completeness of a ten-year (2001 to 2010) daily ground measured global horizontal irradiation dataset provided by the Trinidad and Tobago Meteorological Service, one year (2003) was found to have no missing values. In this study, this short-term one-year dataset was used to correct two long-term freely available modelled datasets using a measure-correlate-predict technique by developing a linear relationship between the measured and modelled data. The corrected modelled datasets exhibit a reduction in bias, outliers, and variance. From these corrected datasets, the inter-annual variability was determined to be small, with coefficients of variability ranging from 2.1 to 2.4 %. Thus, short periods of measurements (1 year) could potentially be used to explain the long-term characteristics of the solar resource in Trinidad and Tobago. Also, low inter-annual variability of the solar resource in Trinidad and Tobago will correspond to low inter-annual variability of solar power generation derived from the resource, and thus, allows for greater predictability in annual returns on investment.

Keywords: Solar Resource, Global Horizontal Irradiance, Measure-Correlate-Predict, Inter-Annual Variability, Trinidad and Tobago, and PV

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Introduction

As of 2022, the Government of the Republic of Trinidad and Tobago has plans to install their first grid-scale photovoltaic systems (The Energy Chamber of Trinidad and Tobago, 2020). Due to large financial investments, investors and project developers need a clear understanding of the short-term and long-term risks and uncertainties associated with their investment. As a means of risk evaluation, in the early stages of project development, solar project developers consider the feasibility of photovoltaic projects which can guarantee a reliable estimate of the system performance during the project life (Holler et al., 2021). In feasibility assessments, the accuracy of the solar resource information and the variability of the solar resource itself directly impact system performance estimates and thus the associated cost of the generated electricity (Meyer and Sengupta, 2016). For example, an overestimation of the solar resource will result in an overestimation of the estimated energy yield. If the actual energy yield does not meet the initial estimates, less revenue from energy sales will impact the servicing of debt or investment returns (Tjengdrawira and Richter, 2016). Hence, the use of site-representative, long-term solar resource datasets which reflect site-specific trends and inter-annual variability of the solar resource will improve solar energy assessments (Coker, Barlow, Cockerill & Shipworth, 2012; Engeland et al., 2017).

In order to support the integration of renewable energy systems, the Inter-American Development Bank and The European Union supported consultancies to develop a renewable energy roadmap and framework for Trinidad and Tobago between 2015 and 2017 (Marzolf et al., 2015; Stefanou, 2017). The studies evaluated the technical performance of PV systems and the associated levelized cost of energy (LCOE) of solar projects. The modelled data used, GAISMA and World Bank SolarGIS, have not been site validated for Trinidad and Tobago (Marzolf et al., 2015; Stefanou, 2017; GAISMA, n.d.; Stackhouse et al, 2015; PANGAEA, 2021) or bias-corrected using high-quality ground-measured data which can lead to large uncertainties. For example, the global horizontal irradiance (GHI) uncertainty in the World Bank SolarGIS data for equatorial regions of America and in regions with limited or no availability of high-quality ground measurements can be as high as ± 8 % (ESMAP, 2019). Modelled datasets that have not been site-compared and corrected have unquantified uncertainties and can introduce a large risk in the photovoltaic project and can compromise investments. It is recommended that at least one year of high-quality ground measurements should be collected to determine the accuracy of modeled datasets (Ramírez, 2017).

Thus, the objective of this study is to decrease the uncertainty associated with long-term modeled daily global horizontal irradiation for Trinidad and Tobago by evaluating and correcting its site-specific bias and then further evaluating its long-term inter-annual variability. In section 2 we briefly describe the ground measured and modeled solar irradiance datasets used, and the methods to evaluate site-representativeness of the data, correct the modeled datasets via a relationship with the ground data, and analyze the inter-annual variability of the corrected datasets. In section 3 we present and discuss the main findings, comparing the site-representativeness of the two modeled datasets, how they improved after correction and their associated long-term inter-annual variability. In section 4 we conclude with the main findings and include associated future work which is currently being undertaken.

Methodology

Ten years of daily on-site global irradiation data (2001–2010) were obtained from the Trinidad and Tobago Meteorological Service. Data for 2003 contained no missing values and was therefore used to evaluate the representativeness of two long-term modeled datasets, NREL's PSM V3 (U.S. Department of Energy, 2022) and NASA's SRB v3.0 (NASA, n.d.). The existing bias was corrected using a measure-correlate-predict (MCP) method (Tahir et al., 2021) for the overlapping period between the ground measured and modelled datasets. Since NASA's SRB v3.0 dataset ended in 2007, the correction for this dataset was done for 2001 to 2007, while the correction NREL's PSM V3 dataset was applied for the period 2001 to 2010. The corrected datasets were also compared to the existing incomplete ground measured data. The inter-annual variability of the corrected long-term modelled data was then assessed using the coefficient of variability indicator (Kariuki & Sato, 2018).

The evaluation of the representativeness of the long-term modelled data was done through the analysis of scatterplots and three indicators of dispersion, namely, the mean bias error (MBE), root mean square error (RMSE) and the mean absolute error (MAE) (Salazar et al, 2020; Escobar et al, 2014; Gueymard, 2014;

Gueymard and Myers, 2008; Gueymard and Wilcox, 2011; Almeida, Perpiñán and Narvarte, 2015; Coimbra, Kleissl and Marquez, 2013). Using a scatterplot of modeled vs measured irradiance at a site, a slope of best fit and the coefficient of determination, R^2, was obtained. In addition, the visual scatter indicates random errors (Gueymard and Myers, 2008) while the bias indicates systematic errors (Gueymard and Myers, 2008) in the modelled dataset. The R^2 value shows how well the modelled data predicts the trends in the measured data (Coimbra, Kleissl and Marquez, 2013).

The MBE is commonly used to quantify the bias between the modelled data and the measured data (Gueymard and Myers, 2008; Almeida, Perpiñán and Narvarte, 2015; Coimbra, Kleissl and Marquez, 2013; Harmsen, Cruz and Mecikalski, 2014; Riihelä et al, 2015; Ordonez, Vaca-Revelo and Lopez-Villada, 2019). The MBE indicator, as a percentage, is defined as

$$MBE = \left(\frac{100}{O_m}\right)\frac{1}{N_p}\sum_{i=1}^{i=N_p}(p_i - o_i) \qquad \text{(eq. 1)}$$

where is the mean of the values in the observed data distribution, is the number of points, is the i^{th} predicted or modelled data point and is the i^{th} observed data point.

The RMSE is used to evaluate the variance of the difference between the modeled data and the measured data (Coimbra, Kleissl and Marquez, 2013). The RMSE indicator, as a percentage, is defined as

$$RMSE = \left(\frac{100}{O_m}\right)\left[\frac{\sum_{i=1}^{i=N_p}(p_i - o_i)^2}{N_p}\right]^{\frac{1}{2}} \qquad \text{(eq. 2)}$$

The MAE is useful in unveiling outliers (Almeida, Perpiñán and Narvarte, 2015). The MAE indicator, as a percentage, is defined by

$$MAE = \left(\frac{100}{O_m}\right)\sum_{i=1}^{i=N_p}|p_i - o_i| \qquad \text{(eq. 3)}$$

The biases associated with the modelled datasets, NREL's PSM V3 (2001 to 2010) and NASA's SRB v3.0 (2001–2007), were corrected using a MCP approach by developing a linear relationship between the measured and modelled data for the year 2003 as shown in equations 4 and 5 (Tahir et al, 2021):

$$H_{m,i} = m\,H_{e,i} + c \qquad \text{(eq. 4)}$$

$$H_{ec,i} = m\,H_{e,i} + c. \qquad \text{(eq. 5)}$$

where $H_{m,1}$ is the measured daily global horizontal irradiation, m is the gradient obtained from the linear relationship, $H_{e,i}$ is the estimated daily global horizontal irradiation from the modelled datasets, c is the y intercept obtained from the linear relationship, and $H_{ec,i}$ is the corrected daily global horizontal irradiation.

The inter-annual variability is determined by use of the coefficient of variability (COV) (Kariuki and Sato, 2018):

$$COV_{time} = \frac{\sigma_t}{H_T} \times 100 \qquad \text{(eq. 6)}$$

where

$$\sigma_t = \sqrt{\sum_{t=i}^{k} \frac{(H(t)-H_T)^2}{k}} \qquad \text{(eq. 7)}$$

where is the standard deviation of the inter-annual variation, is the annual average of the mean, is the annual mean for each individual year t, and k is the total number of years.

Results

The NREL PSM V3 dataset was found to be more representative of the ground measured solar resource at the site compared to NASA's SRB v3.0 dataset. The NREL PSM V3 dataset was found to have fewer random errors as its scatterplot showed less scatter (Figure 1), smaller bias due to its smaller MBE of 25.0 % compared to 36.6 %, fewer outliers due to its smaller MAE of 25.3 % compared to 37.5 %, and smaller variance due to its lower RSME of 26.7 % compared to 40.6 % (Table 1).

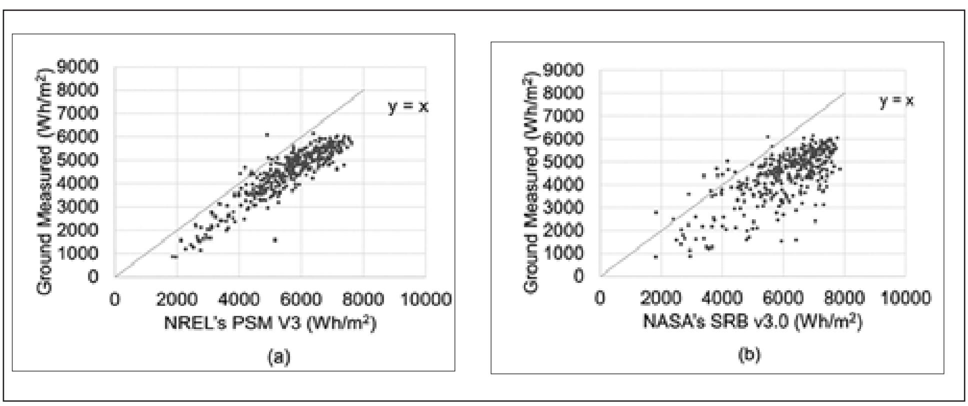

Figure 1: Scatterplots of the daily ground measured GHI totals compared to the modelled datasets for the year 2003

Table 1: MBE, MAE and RSME (%) of modelled datasets before and after MCP correction

Statistical indicators	Dataset			
	NREL's PSM V3		NASA's SBR v3.0	
	Before Correction	After Correction	Before Correction	After Correction
MBE (%)	25.0	-1.0	36.6	0.3
MAE (%)	25.3	10.0	37.5	-3.4
RMSE (%)	26.7	3.6	40.6	6.9

On applying the MCP correction using equations 4 and 5, to both datasets and comparing them to the existing ground measured daily dataset, a significant reduction in bias, outliers, and variance is observed (Table 1). The gradient and intercept coefficients of the MCP correction are listed in Table 2.

Table 2: Coefficients m and c obtained from applying the MCP method

MCP model coefficients	Dataset	
	NREL's PSM V3	NASA's SRB v3.0
Gradient, m	0.8298	0.6467
Intercept, c (Wh/m^2)	-165.5	510.6
R^2	0.8250	0.5094

After correction, the MBE, MAE, and RSME for the corrected NREL's PSM V3 dataset for 2001 to 2010 reduced to -1.0 %, 10.0 %, and 3.6 % respectively. For the corrected NASA's SRB v3.0 dataset for 2001 to 2007, the MBE, MAE, and RSME reduced to 0.3 %, -3.4 %, and 6.9 % respectively (Table 1).

The inter-annual variability of the corrected datasets indicated low inter-annual variability in the solar resource as a COV of 2.4 % and 2.1 % were found for NREL's PSM V3 and NASA's SRB v3.0 datasets, respectively. Since the inter-annual variability is low, short periods of measurements could potentially be used to explain the long-term characteristics of the solar resource (Kariuki and Sato, 2018).

Conclusion and Future Work

The modelled data showed significant site-specific biases that could introduce large risks in estimates of photovoltaic system performances. After correction, a significant reduction in bias, outliers, and variance was observed. The correction will lead to a reduction in the overestimation of the resource, reducing the uncertainty associated with underestimating the associated LCOE. In addition, since the inter-annual variability is low, short periods of measurements may be useful in characterizing the long-term solar resource in Trinidad and Tobago.

Currently the authors are working on creating long-term, sub-hourly, direct normal, global horizontal, and diffuse horizontal solar resource datasets done by establishing a solar resource monitoring network in Trinidad and Tobago to collect updated global horizontal, diffuse horizontal and direct normal irradiance data at hourly and minutely temporal resolutions for one year. The measured data will then be used to evaluate the representativeness and site-correct existing long-term modelled datasets. Sub-hourly and minutely measured data provide a unique opportunity to capture the sub-hourly weather variability of solar irradiance, and relationships between global irradiance and its direct and diffuse components at a higher resolution. Understanding the solar resource on sub-hourly scales will assist in improving the estimates of the energy yield of PV systems.

Acknowledgements

Sincere thanks to the Trinidad and Tobago Meteorological Service for providing data for this project.

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Journal of Arts, Science and Technology

To be or not to be

Inducers, Inhibitors and Implications for Transport Decarbonisation in a Small Island

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Abstract

The Barbados National Energy Policy (BNEP) 2019-2030 articulates the ambition of a 100% renewable energy economy and the Nationally Determined Contribution commits to a "more efficient, reliable, affordable and resilient" transport system, all by 2030. Currently, Barbados' transport sector is dominated by private car ownership, low use of shared modes and reinforcing norms. However, only three of 22 BNEP activities have been implemented as planned by 2022.

This paper investigates whether sector experts consider the sustainable decarbonisation of the Barbados transport sector by 2030 to be viable, their supporting reasons and the potential policy implications. A Delphi expert study was used to obtain perspectives from professionals in road transport and related sectors in Barbados. An inductive thematic analysis was applied followed by a deductive analysis using an expanded avoid-shift-improve framework (ASI+).

It found that experts were evenly divided about whether the 2030 decarbonisation is feasible. Financial incentives and investment, regulation and enforcement, stakeholder and political buy-in were the greatest enabling factors identified; while inappropriate regulation and legislation, inadequate financing, unaffordability and local unavailability of electric vehicles were seen as the greatest inhibitors. Most enablers related to improving technology and efficiency, finance and governance. Similarly, 83.3% of BNEP measures directly apply to the improve segment of the ASI framework. The literature, however, frequently finds that avoiding travel demand and shifting to more efficient modes are more *effective at reducing emissions. Given this, the sector is likely to continue reinforcing the business-as-usual car-dependence paradigm, making the intended goal an improbable prospect.*

Keywords: Decarbonisation, Sustainable Transport, Delphi, ASI Framework, Transport Policy, Barbados and Small Island

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Introduction

Barbados is an independent small island developing state (SIDS) in the Caribbean, dependent on fossil fuel imports for most of its electricity, transport (road, maritime and aviation), residential and industrial needs. In 2019 this amounted to 3.89 million barrels, declining to 3.31 million barrels during 2020 with the outbreak of the COVID-19 pandemic and an associated 5% fall in electricity demand. This further fell to 3.11 million barrels in 2021 (MEWR 2020; MESBE, 2021). Fuel consumption by the transport sector makes it the second highest greenhouse gas (GHG) emitter after electricity generation at 33% (Government of Barbados, 2019).

The government articulated its ambition in the Barbados National Energy Policy (BNEP) 2019–2030 to become a 100% renewable energy economy by 2030 (Government of Barbados 2019). Its updated Nationally Determined Contribution (NDC) solidifies this as an international commitment, promising a 95% renewable energy mix and 100% alternative fuel or electric vehicles (EVs) in the passenger fleet (Government of Barbados, 2021), with the objective of achieving a "more efficient, reliable, affordable and resilient" transport system (Government of Barbados, 2021 p.16). Despite this, a series of 22 activities planned for the first three years within the BNEP Implementation Plan are still incomplete. The only measures to come to fruition have been the introduction of electric buses to public transport, and in the most recent March 2022 national budget, a 2-year waiver on excise and value added taxes (VAT) on electric vehicles along with an announced road usage tax for electric vehicles based on vehicle-km.

To date, there is no overarching strategy for the management of the transportation sector, though national consultations were held in 2019. The draft Physical Development Plan (PDP) (Government of Barbados, 2017) supports a green economy transition which includes advancing mobility and accessibility through prioritising alternatives to private vehicles, including multimodality, active, public and water transportation as well as parking management. The PDP foresees the integration of the sector with land use planning to reduce travel demand and to enhance connectivity between rural, suburban and urban areas, and acknowledges the role of transport in climate change mitigation and hazard resilience. The BNEP expresses the intention to discontinue the use of gasoline and diesel in the local road transportation market through electrification, use of biofuels and efficiency improvements (Government of Barbados, 2019). It recommends specific measures including greening public transport; switching to ethanol and biodiesel; eliminating VAT and duties on electric vehicles; and incentivising investment in charging stations.

An estimated 20% of the local population was dependent on public transport a decade ago (Robinson 2012). The government-operated Transport Board met 60% of the demand and the remaining 40% by privately-owned public service vehicles (PSVs), though by 2019 registered PSVs reached 782 compared to the Transport Board fleet of 69. Available data show significant declines in Transport Board annual bus ridership since the 1980s peak, particularly during 2018–2019 (discounting 2020–2021 when bus ridership was actively restricted as part of COVID-19 pandemic containment measures); however, such data are not centrally captured for the independent PSVs. Trends suggest an inverse relationship between GDP per capita and public transport use (Figure 1), also seen in the literature (Gascon et al., 2020; Girod, van Vuuren, and de Vries, 2013). Rise in car ownership with increasing income is shown globally to be a key factor in decreased public transport use (Buehler, 2011; Gascon et al., 2020; Girod, van Vuuren, and de Vries 2013; Sultan, 2015).

Barbados has a fleet of 135,742 registered vehicles (2020), or 472 vehicles per 1,000 persons, which grew from approximately 93,000 in 2002 and 126,000 in 2008. Of these, 95% are light duty vehicles (LDVs) i.e. cars and other light 4-wheeled vehicles, motorised 2 and 3-wheeled vehicles (da Silveira 2020). The fleet is 85.6% gasoline-driven and 14.2% diesel. By mid-August 2022, Barbados had the second highest average gasoline prices in the world at US$2.39/l[1] and eighth for diesel at US$2.12/l[2]. The government subsequently instituted a temporary cap on petroleum prices in that month at US$2.24/l on gasoline and US$2.01/l on diesel (Austin, 2022).

Local data show SUVs and pickups increasing in proportion of sales during 1999–2020, being second and fourth highest on average among vehicle types at

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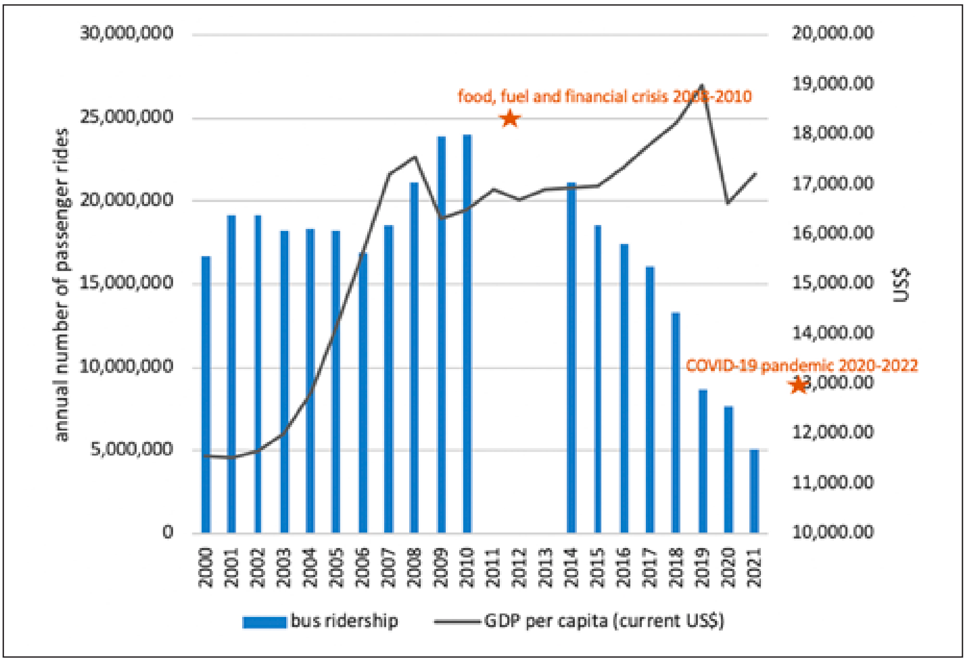

Figure 1: Trends in bus ridership and GDP per capita in Barbados
(*Sources:* Barbados Transport Board, Transport Authority, World Bank)

24% and 14% respectively. They further rose during 2016–2020 to first and third at 40% and 17.5% respectively, with hatchbacks at 18.5% (Figure 2). This is replicated in the EV market, with over 100 SUVs being sold in their first year of introduc-

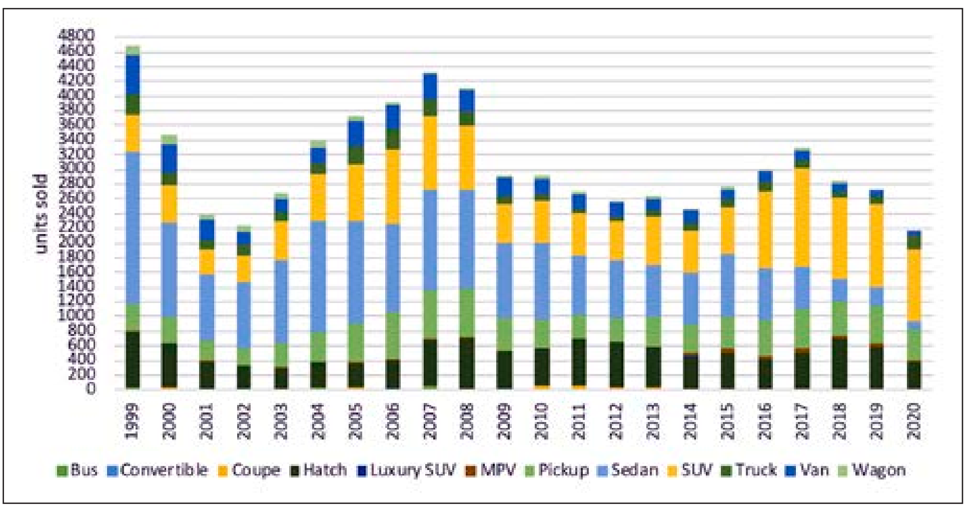

Figure 2: Vehicle sales by manufacturer-licensed dealerships in Barbados by type
(*Source:* Barbados' Dealer Automotive Group)

tion in 2020 during the COVID-19 pandemic, compared to electric car sales of about 500 since 2013 (Edghill, 2021). This appears to mirror the trend in Europe, Asia and the USA towards owning larger, less efficient private vehicles (Banister, Pucher, and Lee-Gosselin, 2007; Griffiths, Furszyfer Del Rio, and Sovacool, 2021; IEA 2020b; Moriarty and Honnery, 2019).

Notably, Barbados has the most EVs in the Caribbean at over 650 private and government vehicles (Edghill, 2021; Viscidi et al., 2020). This is in addition to the electric bus fleet commissioned in August 2020 which reached a total of 49 within a year (Madden, 2021). 2020–2021 saw the most significant growth with over 150 EVs added to the roads. However, in total, this represents about 0.5% EV penetration.

The purpose of this paper is to investigate why the transport sector has not seen substantive traction in its transformation, despite high-level commitments and ambitious goals. Such limited progress presents a challenge for meeting the transition targets in less than a decade, particularly as vehicle registrations and transport demand continue to grow. With a rapidly closing window to transform an expansive and high-emitting sector, which engages almost every member of the population, the ability to identify challenges, pivot and adapt will be significant to maintain hope of its realisation. Thus, the study asks whether experts consider the sustainable decarbonisation of the Barbados transport sector by 2030 to be a viable undertaking, the reasons supporting their perspectives and resulting potential policy implications.

Literature Review

Seto et al. (2016) described three interrelated and deeply entrenched types of carbon lock-in which exercise inertia against system-level shifts, such as Barbados' sector transformation. Infrastructural and technological lock-in describes the high investment costs and long lifetimes concerned with the built environment, which drive transport mode choices, travel distances and behaviours. Institutional lock-in reinforces the former through purposefully designed structures and decisions by political, economic and social actors to support goals and interests for an intended end or desired status quo, even despite suboptimal welfare outcomes. Behavioural lock-in describes how lifestyles, habits, norms and preferences shape consumption patterns (Seto et al. 2016).

The avoid-shift-improve (ASI) framework (Banister, Pucher, and Lee-Gosselin, 2007; Enriquez et al., 2014) is widely used in global analyses on improving environmental sustainability and mitigating climate change (IEA, 2020b; Sims et al., 2014; UNEP, 2020). It describes three primary types of actions that can be taken to reduce GHG and lifecycle emissions (not limited to transport). Actions in each category address at least one type of carbon lock-in.

Avoiding (or reducing) the need to travel arguably has the greatest emissions reduction potential, on average 3.7tCO_{2e} per capita annually for road transport (UNEP, 2020). Aside from climate change mitigation, reducing vehicular travel, particularly in urban areas, boasts multiple co-benefits, such as reduced congestion, noise and air pollution; fewer road accidents; increased exercise through non-motorised transport (NMT) or active modes; shorter (or avoided) commutes and increased productivity; reduced geographical sprawl; less land use for parking and vehicle infrastructure and more green space options (Moriarty and Honnery, 2019; Thaller et al., 2021; Enriquez et al., 2014; Brand, Anable, and Dixon, 2020; Brand et al., 2020; Ogilvie et al., 2004). Shifting to (or maintaining) transport modes with higher energy efficiency emphasises increasing public transport (bus, bus rapid transit (BRT), light rail, tram, etc.) and other shared modes (carpooling, ridesharing, etc.), which have lower emissions per passenger km due to their high occupancy levels. Improving technology includes efficiency improvements and switching to cleaner and more efficient fuel and vehicle technologies (including hybrids, electric, biofuels and hydrogen), while maintaining the consumption activity. De Blas et al. (2020) note that many models of a 100% transition of transport to renewable energy rely on expansion of prospective fuel technologies such as hydrogen and biomethane. However, the timeframe within which these will become commercialised is highly uncertain.

Methodology

A Delphi expert study (Melander, 2018; Stephenson et al., 2018; Varho and Tapio, 2013; Zartha Sossa, Halal, and Hernandez Zarta, 2019) was conducted with experts in road transport and related sectors in Barbados. It adopted an approach based on Stephenson et al. (2018), which similarly sought to identify factors shaping the transport system, interventions needed and barriers to a sustainable transition. This study applied it to a small island context.

Invitations were extended to capture every key stakeholder in sector planning and management – central government agencies responsible for transport infrastructure, energy, environmental regulation, customs and planning, state-owned entities (SOEs) responsible for public transport, the lone electric utility, the national oil distribution company, major automotive dealers and the associations for renewable energy, engineers, energy professionals and public transport operators. Academia and develokpment organisations were also invited due to their active roles in research and development, policy and technical assistance for the local sector.

Consensus building was not the objective, as is typical of most Delphi studies. Rather, as with Stephenson et al., (2018), the aim was to stimulate a thought process among stakeholders to uncover the factors perceived to be affecting progress of the sustainable transition of the sector to the desired state, and key actions to advance that process.

Three rounds of anonymous online surveys were conducted in January 2022, given COVID-19 restrictions, with some attrition (30%) as the rounds progressed, as is expected (Hall et al., 2018; Stephenson et al., 2018; Chalmers and Armour, 2019). Government agencies (including SOEs) and private companies together comprised 60% of participants (Figure 3) as expected based on the number of entities which exist within these groupings. On average, participants had 11.7 years of experience in work related to transport (Table 1), where 25% had 20 years or more and 40% had 5 years or less. As is typical in energy and transport-related fields (Battams et al., 2014; Lefrançois and Probst, 2020; T. Wright, 2014; Varho and Tapio, 2013), male representation dominated at 60%.

In Round 1 (n = 20), participants were asked to describe their views on what a sustainable transport system would look like, whether it could be achieved by 2030 and what factors would enhance or hinder this. Round 2 (n = 14) asked them to indicate using a Likert scale their level of agreement with the factors indicated

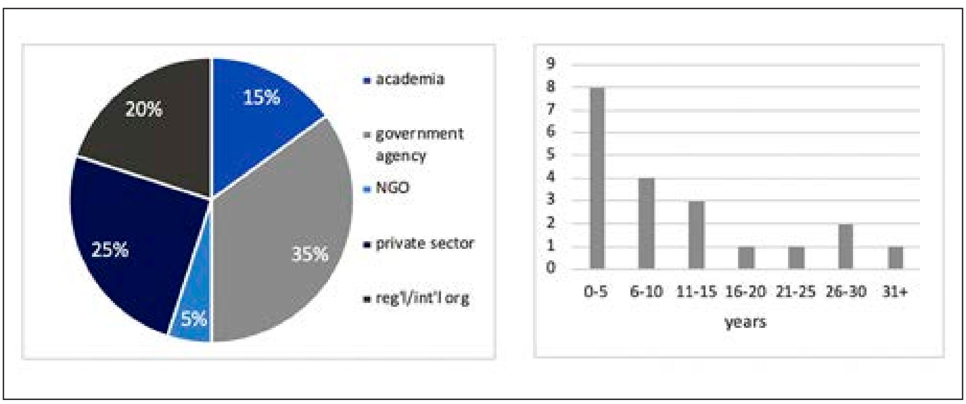

Figure 3: Round 1 participant representation by type of agency (left) and transport sector experience (n = 20)

Table 1: Fields and years of work disaggregated by self-assessed levels of expertise of Round 1 participants (n = 20)

Level of expertise	Primary area of work	Average number of years working in or supporting the transport sector
Expert		*15.2*
	Automotive administration	30.0
	Biofuel production	6.0
	Lecturer	3.0
	Project management and procurement	25.0
	Solar power development	12.0
Seasoned		*18.1*
	Automotive sales and service	30.0
	Business administration	20.0
	Economics	10.0
	Sales, parts, service and administration	37.0
	Sustainable energy	10.0
	Transport planning and engineering	15.0
	Transport policy	5.0
Mid-level		*7.3*
	Energy engineering	5.0
	Engineering management	6.0
	Project management	15.0
	Transport	3.0
New entrant		*0.7*
	Climate change	0.0
	Project management	1.0
	Regulatory affairs	1.0
Overall mean		**11.7**

in Round 1, based on a frequency distribution of the group's responses, and then their agreement with additional factors identified through an international Delphi study (Stephenson et al., 2018). They were then asked to describe the most urgent interventions needed in the sector and their likelihood of success (i.e. scale of adoption, transformative potential and implementation timeframe). During Round 3 (n = 14), they were asked to identify barriers and taboos (i.e. impediments to policy design, acceptance and implementation that remain unaddressed because they represent political risk (Gössling and Cohen, 2014) that would obstruct these changes. This paper focuses on the results of Rounds 1 and 2 with Round 3 to be presented in future papers.

An inductive thematic analysis was undertaken of the qualitative data for the initial coding, and descriptive statistics performed on the emerging quantitative data. Deductive analysis of the described factors and interventions used avoid-shift-improve as themes, supplemented by emergent cross-cutting themes where the described factors were non-specific and could support multiple ASI aspects, such as governance and finance (ASI+). They were simultaneously categorised by types of carbon lock-in being addressed. The factors were further compared to the existing measures described in the BNEP 2019–2030 to determine coherence and gaps between the factors and policies.

Results

Attributes of the Transition and its Feasibility

Efficiency and reliability, lower emissions and pollution, use of clean fuels and affordability were the characteristics most frequently identified in Round 1 as elements that should constitute a sustainable transport system in Barbados (Figure 10). Increased public transit and scalability were the least popular themes, each being mentioned by only one person, while social and economic benefits were in the middle of the distribution. Resilience was the only characteristic from the NDC definition not mentioned. Round 2 saw 71% agreeing or strongly agreeing with the characteristics described, though the list of 15 attributes dwindled to 12 as participants chose their top 5 themes. The top 4 attributes remained in Round 2; but equity, multi-sector planning, social needs and economic benefit dropped substantially in importance. The tax regime was the only item to move upward in the rankings. Moreover, public transit investment, inclusive mobility and user safety fell off the list. Overall, half of the attributes chosen commanded 82.4% of the responses.

The low perception of social elements such as safety, inclusion and increased public transport (which could refer to its accessibility, affordability and/or availability) may indicate diminished importance given by experts to the social pillar of sustainable development as a driver in transport, such as found in a technoeconomic management approach. A technoeconomic approach focuses on

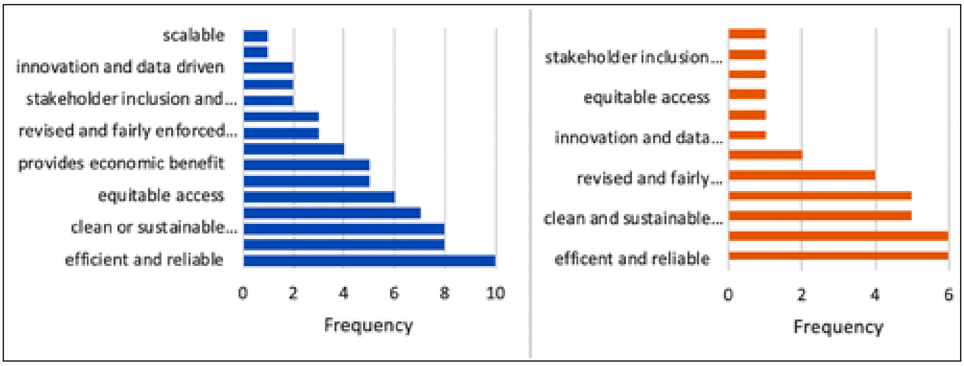

Figure 4: Characteristics of a sustainable transport system in Barbados expected by participants as initially described in Round 1 (left) (n = 20) and following a Likert scale in Round 2 (n = 14)

economic performance based on capital and operating costs, benefits, risks, and uncertainties of technology or process in managing the sector.

Some (Geels et al., 2017; Sovacool and Griffiths, 2020; Brand, Anable, and Morton, 2019) have cited the limitations of this approach in not accounting for several variables that influence travel patterns and energy use outside of technology and pricing mechanisms, and which therefore may generate misleading interpretations. This contrasts with a multidimensional sociotechnical framework which encompasses interlinkages between infrastructure, technology, markets, policies, preferences, behaviour and culture (Anable et al., 2012; Brand, Anable, and Morton, 2019; Geels, 2012; Rip and Kemp, 1998; Geels et al., 2017; Velazquez et al., 2015).

Indeed, literature typically describes travel behaviour as being attributed to the following characteristics to varying degrees: spatial development patterns, socioeconomics and demographics, national culture and individual preference, and direct or indirect policy (Buehler, 2011). A 50:50 divide exists among stakeholders about whether the 2030 vision of a sustainable transport sector is feasible, with 6 males and 4 females selecting both answers. If beyond 2030, it was most considered to be attainable by 2040 (Table 3), even among some who believed 2030 was possible.

On average, the optimists had 8.8 years of experience in transport, while the sceptics had 14.6 years. This may suggest a lack of confidence in the ability to effect change within the existing system among persons who have been entrenched in it longer. Such persons are more likely to have a greater understanding of the system and to have witnessed failed or stagnated change processes, thus being more pessimistic about its potential to adjust.

Table 2: Alternative timelines proposed by participants about when the sustainable transport transition could be realised in Barbados by type of agency represented (n = 20)

Type of agency (n)	2030	2031–2035	2036–2040	2041–2045	2046–2050
Academia (3)	3				
Government agency (7)	3	1	3	1	
International agency (2)	0		2		
NGO (1)	1		1		
Private sector (5)	1	1	2		1
Regional agency (2)	2				1
Total	**10**	**2**	**8**	**1**	**2**

All participants from academia, NGOs and regional agencies considered the transition a plausible prospect by 2030. Conversely 80% of private sector participants and 43% of government representatives did not. This may similarly be reflective of the depth of familiarity and regular interaction with the operational and governance machinery of the transport sector. Locally, academia and development agencies are typically divorced from such mechanisms, providing insight or assistance usually within the context of a discrete work programme adjunct to the sector. PSV associations are among the few civil society organisations that are active stakeholders in transport.

Inducing and inhibiting factors

Those optimistic about 2030 described key factors that would enable this transition (Figure 5). Financial incentives and investment, regulation and its enforcement, stakeholder and political buy-in were the most mentioned among these. More than 50% of the themes had just one mention each, including the need for a plan to address scrapped internal combustion engine (ICE) vehicles, charging infrastructure and an overarching sector strategy.

Sceptics described the key facto rs inhibiting the transition by 2030. Inappropriate regulation and legislation as well as inadequate financing were cited the most, followed by affordability then local availability of EVs. Again, 50% of responses had a single response, including negative impacts on government revenue and employment, and deficiencies in public demand and high-level advocacy.

Participants in Round 2 generally shared these perspectives, with 57% in agreement or strong agreement on the enabling factors and 65% for the inhibiting factors.

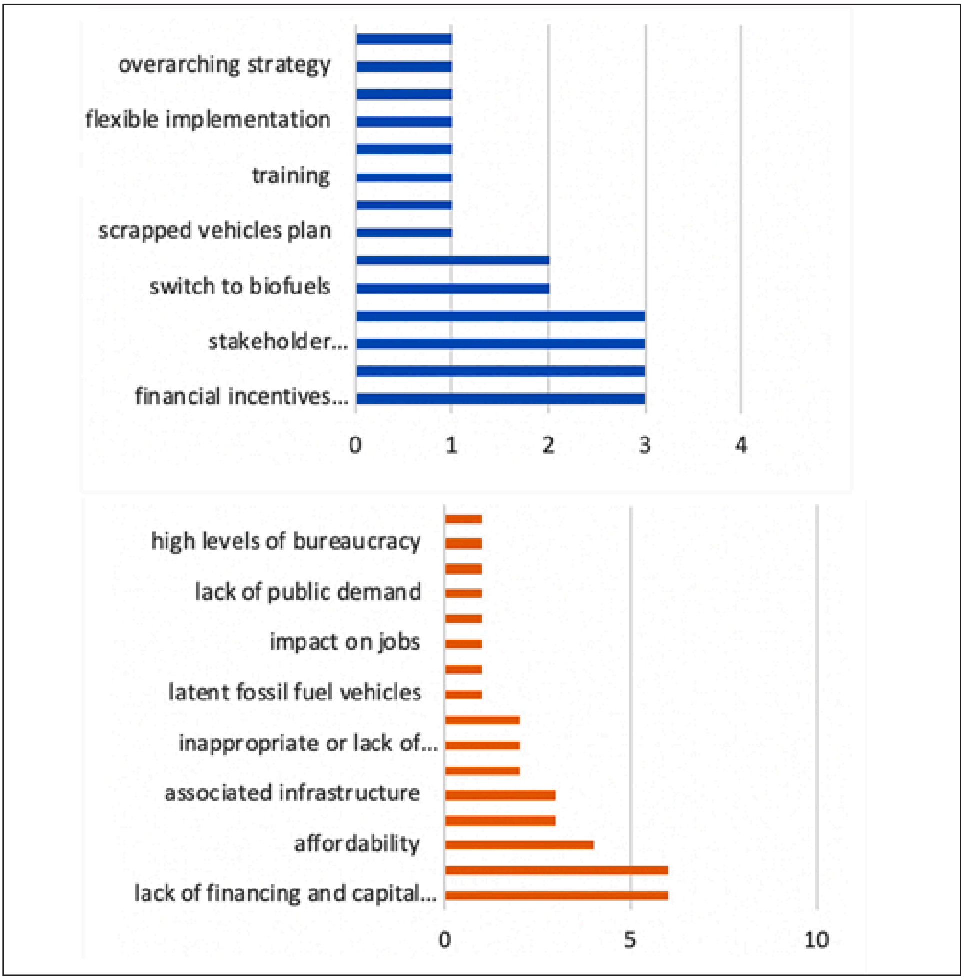

Figure 5: Key factors that Round 1 participant believed would support (left) or inhibit realisation of the sustanable transport transition by 2030 (n = 2)

Disagreement or strong disagreement totalled 21% and 28% toward enabling and inhibiting factors respectively.

Additional factors from the Stephenson et al. (2018) study was posed (excluding those that would be inapplicable e.g., for rail transport) for participants to indicate their local relevance (Table 3).

Table 3: Participants' percentage level of agreement with drivers of the transport sector as identified by international experts in Stephenson et al. (2018) (n = 14)

Inducing factors	Strongly agree	Agree	Neither	Disagree	Strongly disagree
Investment in new technologies and infrastructure	64.29	28.57	7.14	0.00	0.00
Internet access contributing to greater flexibility in work practices e.g. work from home, video conference	42.86	28.57	21.43	7.14	0.00
New mobility-related technologies and business models	35.71	35.71	28.57	0.00	0.00
Travel substitution technologies including ICT	0.00	64.29	28.57	7.14	0.00
New sources of autonomy, freedom and prestige (status)	7.14	14.29	64.29	7.14	7.14
Desire/imperative to address environmental concerns e.g. climate change, poor air quality, congestion, energy security	50.00	50.00	0.00	0.00	0.00
Policy innovations to increase resilience and sustainability in transport systems	71.43	28.57	0.00	0.00	0.00
Outdated laws and regulations for urban planning	35.71	28.57	28.57	0.00	7.14
Vested interests in maintaining the status quo	28.57	42.86	14.29	7.14	7.14
Financial subsidies that encourage oil production and use	28.57	14.29	42.86	7.14	7.14
Lack of political will and leadership	28.57	14.29	28.57	14.29	14.29
Political structure (democratic vs authoritarian)	14.29	7.14	28.57	35.71	14.29
Long-term infrastructure commitments (sunk capital costs)	14.29	57.14	7.14	14.29	7.14
Entrenched beliefs and cultural norms perpetuate "yesterday's" familiar patterns of behaviour, business and governance	21.43	42.86	21.43	14.29	0.00
Work and lifestyle practices requiring individual mobility	28.57	42.86	21.43	7.14	0.00

Table 3: Participants' percentage level of agreement with drivers of the transport sector as identified by international experts in Stephenson et al. (2018) (n = 14) (*cont'd*)

Inducing factors	Strongly agree	Agree	Neither	Disagree	Strongly disagree
Global financial crisis discouraging risk	14.29	14.29	35.71	21.43	14.29
Financial mechanisms	42.86	14.29	28.57	14.29	0.00
Innovations in petroleum extraction	7.69	7.69	38.46	23.08	23.08

Over 60% of participants agreed with all of the driving factors, except one, especially environmental concern and policy innovation at 100%. Most experts (64%) were non-commital about status and autonomy as significant influences in shaping the transport sector. This conflicts with perceived constraints of existing beliefs and cultural norms as well as lifestyles fostering individual mobility (over 60% and 70% agreement respectively) and the existing high levels of car ownership. Such divergences potentially reveal a weak understanding within the sector of the underlying drivers of choices and travel habits, and may suggest policy decisions are biased toward technical and economic parameters, while neglecting sociocultural and behavioural aspects influencing household decisions, among others. This would further be exacerbated by the void of empirical social data on transport in the country.

Outdated spatial planning mechanisms, vested interests, and significant sunk capital costs also found high agreement (over 60%) as inhibitors. This highlights the role of governance in influencing travel behaviour through the built environment, and perhaps the slowness with which such processes change, thus becoming reactive rather than proactively designing the desired future. This is directly linked to private sector decisions which are guided by government policy and regulations in a risk averse climate, and consumer demand which is similarly shaped. However, many inhibitors lacked consensus with widely varying responses, including the influence of oil subsidies, political will, and global crises. This stands against a backdrop of government instituting significant policy changes principally for budgetary reasons, such as removal of fuel subsidies (2014), introduction of fuel tax (2018) and increase in bus fares (2019), which together send conflicting signals for sustainable travel habits or shifting demand, and possibly have exacerbated some social inequalities. Coupled with this is the apparent minimal impact of removing subsidies and increasing global oil prices on the growth of vehicle ownership or fuel consumption. Gasoline prices have increased by 28% over the last 5 years, from an average of US$1.55/l in 2017 to US$2.14/l for 2022 up to August, further compared to US$1.14/l during the 2008 fuel crisis. Vehicle registrations nevertheless increased between 2019 and 2020. This is likely due to a combination of a desire to maintain independent mobility and associated lifestyles, the unreliability and undesirability of public transport, and limited other safe, accessible or affordable alternatives. Data on recent EV sales relative to other fuel types is yet to be seen since the tax waiver and increased fuel prices.

Disaggregating all these factors by the ASI+ themes and type of carbon lock-in revealed a clear preference toward improving technology and efficiency, finance, and governance as enabling factors (Table 4), with emphasis on the institutional

Table 4: Enablers of transport sector transition categorised by ASI+ framework and type of carbon lock-in disrupted from Round 1 (n = 20) and Round 2 (italics) (n = 14)

ASI+ theme	Infrastructural and technological lock-in	Institutional lock-in	Behavioural lock-in
Avoid	*Internet access contributing to greater flexibility in work practices* *Travel substitution technologies including ICT*	*Desire/imperative to address environmental concerns*	–
Shift	*New mobility-related technologies and business models*	Improve public transport operation	–
Improve	Switch to renewable biofuels Charging infrastructure Effective mix of technologies *Investment in new technologies and infrastructure*	Scrapped vehicles plan	–
Finance	Financial investment Public-private partnerships	Financial incentives	–
Governance	–	Regulation and enforcement Stakeholder participation and buy-in Political will and buy-in Flexibility in implementation Overarching strategy *Policy innovations to increase resilience and sustainability in transport systems*	–
Public perception	–	–	Improve public transport perception
Technical capacities	–	–	Training

and infrastructural and technological lock-in components. Several studies agree that deep changes in governance mechanisms are needed for transformation, such as regulatory and financing modalities (Stephenson et al., 2018; Thaller et al., 2021; Wicki, Fesenfeld, and Bernauer, 2019). Adding Stephenson et al. (2018) factors which had 50% or more agreement (italicised) introduced avoid, shift and behavioural lock-in perspectives which were otherwise almost entirely absent.

Conversely, behavioural norms and preferences were recognised more as inhibiting factors (Table 5), though there was still no inclination to move these preferences away from car dependence toward avoid or shift strategies. Respondents with 5 or fewer years in the sector had the most frequent references to behavioural aspects such as “cultural barriers to change”, “ingrained bad habits and indiscipline within the society” and “participation and buy-in of every person” as part of the enabling and inhibiting factors. It was mentioned in only one other instance outside this group. Once more, behavioural lock-in concepts were introduced with the drivers from Stephenson et al. (2018), in avoidance strategies and governance.

The uniformity in types of responses suggests that a greater diversity of stakeholder participation may be warranted in order to generate more varied perspectives and novel ideas. Scholars note that a high degree of experience may be limiting if experts are rooted into a particular way of thinking or planning (e.g. the technoeconomic focus) and may be reluctant to change their views (Winkler and Moser, 2016). Conversely the newer entrants to the sector more frequently described social drivers. The importance of heterogenous perspectives in a visioning process is recognised (Soria-Lara and Banister, 2017; Tuominen et al., 2014; Winkler and Moser, 2016). Soria-Lara et al. (2017) found that the most radical ideas emerged from those 14–35 years old while the oldest respondents tended to have projections more akin to business-as-usual (BAU); they advised that the group composition should reflect the heterogeneity and spatial distribution of relevant actors. The participants who considered the 2030 target to be attainable had an average of 5.8 years less experience. Though they did include academics, lawyers, and natural resource managers, inter alia, none were 35 years or under. Future exercises should therefore seek much greater engagement of the ordinary travelling public to represent diverse travelling experiences and preferences, including youth as future users and decision-makers, innovative thinkers and a demographic with different travel patterns and range of choices than older adults.

Finally, the degree of overlap among policy positions and professional perspectives was examined, comparing the top 5 attributes and enablers identified

Table 5: Hindrances to transport sector transition categorised by ASI+ framework and type of carbon lock-in disrupted from Round 1 (n = 20) and Round 2 (italics) (n = 14)

ASI+ theme	Infrastructural and technological lock-in	Institutional lock-in	Behavioural lock-in
Avoid	–	–	*Work and lifestyle practices requiring individual mobility*
Shift	–	–	–
Improve	Change entire fuel sector Latent fossil fuel vehicles	–	Product (EV) availability
Finance	Lack of financing and capital costs Scalability *Long-term infrastructure commitments (sunk capital costs)*	Government revenue loss *Financial mechanisms*	Impact on jobs Affordability
Governance	–	Inappropriate regulation and legislation Inappropriate or lack of decision making High levels of bureaucracy *Outdated laws and regulations for urban planning*	*Entrenched beliefs and cultural norms perpetuate "yesterday's" familiar patterns of behaviour, business and governance*
Public perception	–		Social habits and behaviours Public demand Lack of high-level advocacy *Vested interests in maintaining the status quo*
Technical capacities	–	–	Lack of human technical capacity

by the experts with the NDC description and the enablers most captured by the BNEP (Figure 6) .

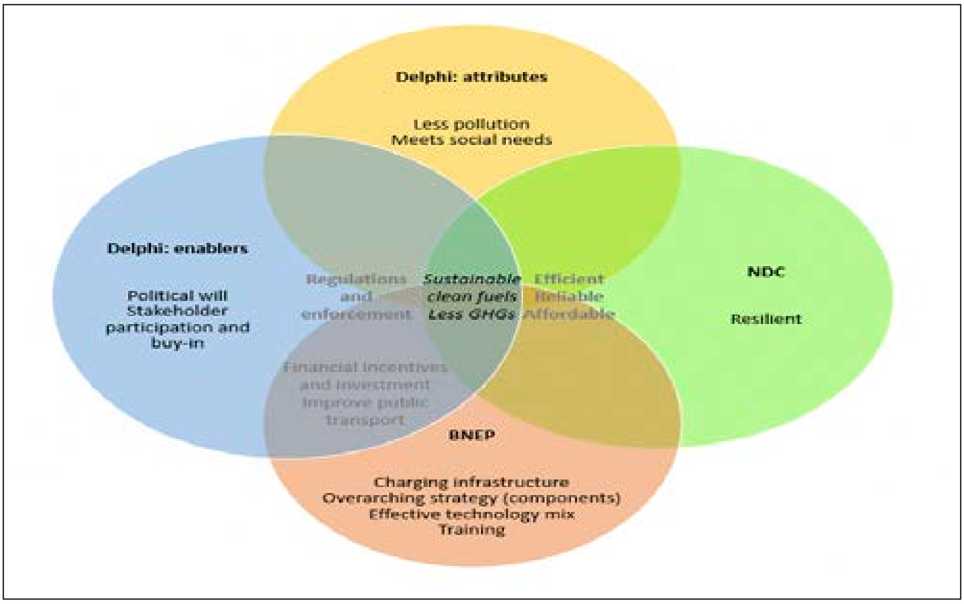

Figure 6: Thematic relationships between the attributes and enabling factors of the decarbonisation transition identified by experts, the attributes within the NDC and measures from the energy policy

Transitioning to clean fuels (and by implication lowering GHGs) was common across the board. A few themes were shared across 3 elements, while financial incentives and public transport were featured in enablers and the BNEP. However, many components did not find coherence across the analysis. For example, activating political and stakeholder engagement is not reflected in the policy. While elements of a sector strategy were itemised in the BNEP, a comprehensive mechanism was not prioritised by the experts, BNEP or NDC. Neither resilience nor social needs resonated across the analysis. This further emphasises the orientation toward a technological rather than mobility transition, concerned with the economics of switching fuels as compared to a holistic approach to ensuring all members of society are able to affordably access transportation to meet their needs, while improving health, social connectivity and environmental wellbeing.

Despite this e-mobility focus, the lack of momentum in the EV transition until recently implies that some factors are weightier than others. For instance, several researchers (Usher et al., 2015; Anable et al., 2012; Barisa and Rošā, 2018; Li, Chen, and Wang, 2017; Sierzchula et al., 2014; X. Zhang et al., 2014) agree that the rate of expansion of charging infrastructure is a significant determinant in the rate of BEV adoption, with range anxiety being a key component of consumer risk. However, despite its small size Barbados has 40 charging locations[3], albeit skewed toward densely populated areas. Fuel prices have been on a general upward trajectory since the 1990s, aside from big dips during the crises of 2009, 2016 and 2020. Excise tax and import duty for EVs and hybrids have been at least 20% lower than ICE vehicles since 2008. However, it was not until the 2-year tax waiver on EVs and reduced tariffs for other alternative fuel vehicles from April 2022 that licensed dealerships officially launched their EV segment and demand significantly outstripped supply from the outset.

Implications for the Transition

Though a developing country and a SIDS, Barbados' transport sector mirrors the transport culture that is prevalent in developed countries (Soomauroo, Blechinger, and Creutzig, 2020; Stephenson, Hopkins, and Doering, 2015), depicted in Figure 7. This might be expected with its classification as a high middle-income country, high human development index (HDI) and service-based economy. Such a culture proves very difficult to escape because its characteristics are extremely

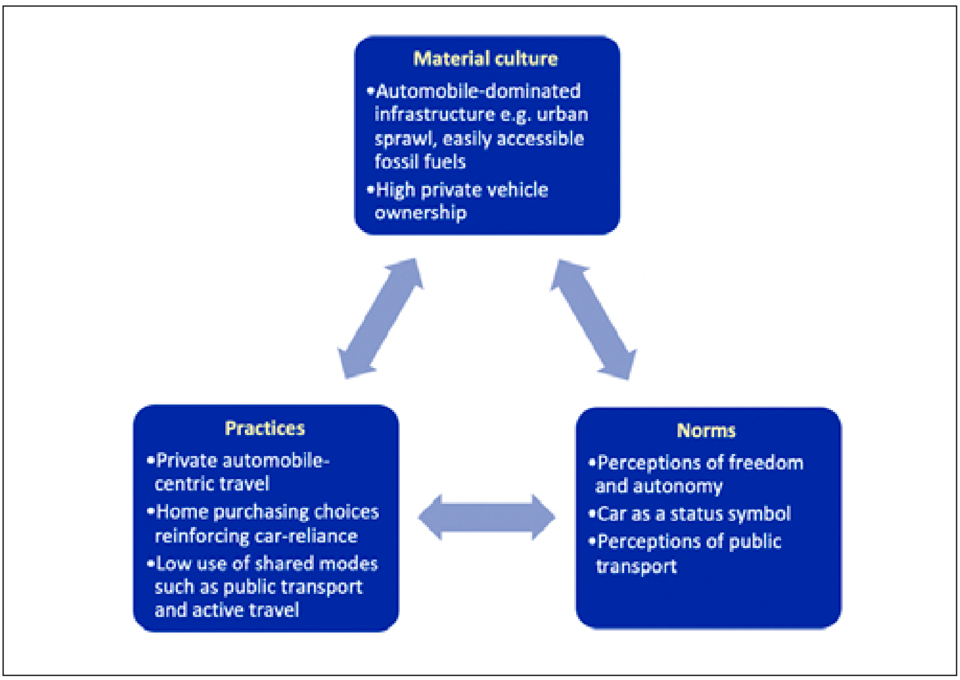

Figure 7: Features of the dominant transport culture (after Stephenson et al., 2015)

3. https://www.plugshare.com/

"interactive, self-reinforcing and self-replicating" (Stephenson et al., 2015 p.359), needing at least one element to be dirupted.

While the BNEP measures and the Delphi findings are broadly complementary in their emphasised themes, together they are asynchronous with the NDC decarbonisation goal based on the factors highlighted in literature as important to driving sector wide GHG emissions reductions. There is widespread agreement that greater weight should not be placed on technology to reduce emissions and increase efficiency, but instead on minimising transport demand (Brand et al., 2020; Creutzig et al., 2018; De Blas et al., 2020; Moriarty and Honnery, 2013; Thaller et al., 2021).

An e-mobility strategy would require replacement of over 13,000 ICE vehicles annually until 2030, assuming no growth in the current fleet. No BNEP measures have been articulated to attempt to restrict demand growth, however. Comparatively, at a derived rate estimated by da Silveira (2020) based on the fleet's age distribution, about 55,000 (40%) of the current vehicle fleet could be replaced by flex fuel vehicles in 10 years. Meanwhile, local experiments showed prospects to use Sargassum seaweed biomethane for 10% of the fleet by converting existing ICEs to compressed natural gas drive trains (Henry et al. 2021). Electric drive train conversions are also possible, though more costly.

Nonetheless, focusing on modal shift within a diverse policy package has been demonstrated to be more cost-effective in GHG reduction than technology improvement (L. Wright and Fulton 2005). Avoid and shift strategies can bring as much as 50% reduction in travel demand compared to lowering carbon intensity of transport fuels (Anable et al., 2012; Girod, van Vuuren, and de Vries, 2013; Salter et al., 2011), with lower inherent environmental risk compared to supply-side solutions (Creutzig et al., 2018). Moreover, many concur that technological substitution will be inadequate both in speed and depth of emissions cuts to reach net zero by 2050 (Rogelj et al., 2018; Creutzig et al., 2018) as sector emissions continue to grow, especially in developing countries. The transition to a sector of mobility requires disrupting the regime with significant sociocultural changes, including reduction in car ownership and use, and similarly adjusted institutional priorities (Köhler, Turnheim, and Hodson, 2020; Pojani and Stead, 2018).

SIDS are characteristically highly vulnerable to external shocks; however, the expert responses neglected such factors as potentially accelerating or constraining the transition. Global oil prices, a major determinant in the cost of transport and imports, saw an approximately US$120 increase for a barrel of crude oil since the April 2020 low until the time of the Delphi exercise. In 2021, though the energy sector experienced a 37.5% decrease in fuel consumption, the cost of imports increased by US$80.9M, a 46.4% increase from 2020 (MESBE, 2021). Continued volatility is a significant push factor for transitioning from fossil fuels, which would improve energy security through renewable energy. Renewables also facilitate a clean EV fleet, which can also provide energy storage through smart charging (Soomauroo, Blechinger, and Creutzig, 2020), and without which market dominance of EVs will increase overall GHG emissions (Fulton, Jaffe, and McDonald, 2019; R. Zhang and Fujimori, 2020).

The deep economic impact from COVID-19 significantly affected government's revenues and debt profile, private sector productivity and profitability, global trade and supply chains, inter alia. With all major inputs for the sector being externally sourced, even with economic recovery, the fiscal constraints in both the private and public sectors would likely affect their ability to manoeuvre into new market segments to some degree. Respondents also overlooked the opportunities presented by this crisis to capitalise on some new practices created, such as teleworking and expanded e-commerce, to lower transport demand and shift norms in the long-term at a system level (Griffiths, Furszyfer Del Rio, and Sovacool, 2021; IEA, 2020a).

Also not contemplated was the intended ban on the sale of new ICE vehicles in several large markets such as Austria, India, Israel (2030), the UK, China (2035), France, Egypt, Denmark and Taiwan (2040) (Burch and Gilchrist, 2020; Fleming, 2020). This will influence the type of automotive stock available for import as EV demand increases, with the possibility of old ICE stock being "dumped" in less stringently regulated developing country markets such as Barbados, as occurs with many consumer goods (L. Wright and Fulton, 2005). De Blas et al. (2020) further reflect on the eventual limitation of critical raw materials, such as lithium and cobalt for EV batteries, inherent with proliferation of current low-emission vehicle technologies. They highlight the importance of vastly improving the materials recovery and recycling of these minerals for the long-term success of a highly electrified transport sector. These should be significant considerations in Barbados' strategy for transport electrification, given the absence of mineral resources and influence on international markets.

The lack of exploration of these and other exogenous drivers and future risks to the economy, society and transport sector more directly should prompt further probing of whether the existing policy is properly fit or sufficiently agile for the current and evolving environment. Further work should examine the policy's relevance in a landscape of systemic and compounding risks.

Seto et al. (2016) determined that a disproportionately large effort is demanded to destabilise all of the carbon lock-in components and shift the dynamics of personal choices and behaviour, with cross-sectoral cooperation to mobilise actors across all spheres for an aggregate positive impact. The perspectives of the experts and the BNEP as presently constructed, particularly noting Table 5 and Table 6, reinforce rather than disrupt the dominant transport culture, and thus reaching the 2030 goal would be considered unlikely.

Study Limitations

Initially, an 8-year experience criterion was used to define "expertise", however, the participants included several below this threshold. It was observed that self-definition of level of expertise in some instances belied the number of years in transport (see Table 2), suggesting that persons (such as academics) were well-established in their fields but perhaps only recently applied their expertise to transport. Therefore, all responses were included in the results, further noting that some stakeholders may otherwise have been unrepresented, given limited personnel within the overall organisation. The definition and value of expertise in such studies continues to be a matter of debate (Mauksch, von der Gracht, and Gordon, 2020).

Access to robust official datasets is a perennial challenge in SIDS for many reasons, including reluctance to share, incompatible formats, inconsistent data collection and a general dearth of data sharing and management policies. Deficiencies in time series related to bus ridership and vehicle registration constrained the ability to investigate correlations between income, car ownership and public transport use, as well as elasticities in demand due to fuel price. No evidence has been found to date on local travel habits and preferences, which would allow identification of patterns among various demographic groups and more concrete analysis of sociocultural influences on choices. Such research will be the subject of forthcoming study. More diverse perspectives in future exercises will also be included.

Conclusion

This study queried whether the decarbonisation of the Barbados transport sector by 2030 is perceived by sector experts to be a viable undertaking, their supporting reasons and potential policy implications. It revealed divided opinions among practitioners about the feasibility of this ambition, with regulation, financing and political will among some of the most common drivers identified. This uncertainty is buttressed by limited implementation progress of planned actions and a guiding policy which seems to perpetuate the individualistic travel patterns and behaviours associated with the BAU car-dominant transport culture.

In contrast, the literature frequently finds that avoiding (or reducing) travel demand and shifting to more efficient modes such as public transport are more effective at reducing emissions than improving technology and fuel efficiency alone, besides many other co-benefits. Addressing governance and finance gaps and opportunities highlighted by sector experts can support transformation across all the ASI segments if appropriately designed and integrated. However, most BNEP actions, inducing and inhibiting factors identified tend toward transport electrification and alternative fuel substitution, and rarely address the need to avoid or shift travel demand.

Barbados exhibits a transport culture more akin to a developed country than a small island, with high private vehicle ownership and low use of shared and active modes. Given the narrow focus of expert perspectives and the BNEP primarily on technological substitution, it is likely that the sector will continue reinforcing the BAU paradigm of travel behaviour. This would therefore be unlikely to disrupt the inertia of carbon lock-in associated with current practice and trends, thus making the intended goal an improbable prospect. More research is needed on travel demand, habits and sociocultural influences on transport choices to design measures that target underlying drivers of behavioural change.

Acknowledgements

I would like to acknowledge and give tribute to the Caribbean's and SIDS' foremost climate change negotiator, Dr Hugh J. Sealy, who started me on this journey but, on account of his passing, was unable to see its completion. Rest well, friend.

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Journal *of* Arts Science *and* Technology

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The *Journal of Arts Science and Technology (JAST)* is the flagship, international, multidisciplinary, peer-reviewed journal of the University of Technology, Jamaica. Its publication is in keeping with one of the objects of the University of Technology Act, to "preserve, advance, and disseminate knowledge through teaching, scholarship and research . . . and to make available the results of such … to promote wisdom and understanding."

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THE UNIVERSITY OF TECHNOLOGY, JAMAICA

Published by
The University of Technology, Jamaica
237 Old Hope Road
Kingston 6, Jamaica, West Indies

ISSN 0799-1681