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About the Nigerian Academy of Science

The Nigerian Academy of Science (NAS) is the foremost independent scientific body in Nigeria, which was established in 1977, and incorporated in 1986. NAS is uniquely positioned to bring scientific knowledge to bear on the policies/strategic direction of the country and is also dedicated to the development and advancement of science, technology, and innovation (STI) in Nigeria. The aims and objectives of the Academy are to promote the growth, acquisition, and dissemination of scientific knowledge, and to facilitate its use in solving problems of national interest. The Academy strives to do this by:

- Providing advice on specific problems of scientific or technological nature presented to it by the government and its agencies, as well as private organizations
- Bringing to the attention of the government and its agencies problems of national interest that science and technology can help solve
- Establishing and maintaining the highest standards of scientific endeavours and achievements in Nigeria, through the publication of journals, organization of conferences, seminars, workshops, and symposia, recognition of outstanding contributions to science in Nigeria, and the development of a working relationship with other national and international scientific bodies and academies

As with national academies in other countries, NAS is a not-for-profit organization with a total membership (since inception) comprising 248 Fellows, elected through a highly competitive process, who have distinguished themselves in their fields, both locally and internationally. Some of her members have served as Vice-Chancellors of universities, Directors-General of government Parastatals and Ministers in federal ministries. The Academy, given its clout, also has the ability to attract other experts from around the country and internationally when needed.

NAS is Nigeria's national representative on such bodies as the International Science Council (ISC) – the umbrella body for all science associations and unions – and the Inter-Academy Partnership for Policy (IAP) – the umbrella body for all national science academies globally. The Academy is also a member of the Network of African Science Academies (NASAC).

Regionally, the Nigerian Academy of Science is one of eight founding academies of the Network of African Science Academies (NASAC) and has served on its Executive Committee till date. The Academy has played a major role in the development and establishment of academies in Africa. In November 2012 and also 2017, the Nigerian Academy of Science hosted the African academies for the 8th and 13th Annual Meeting of African Science Academies (AMASA), in Lagos and Abuja respectively. The Nigerian Academy has signed agreements with counterparts in many African countries (and beyond) to ensure scientific exchange and partnership.

As the peak independent scientific body in Nigeria, the Academy serves as the umbrella body for all science associations in the country, speaking for the same within and outside the country. The Academy holds periodic meetings with representatives of the associations to discuss the state of science in Nigeria and proffer solutions for improvement.

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The *Proceedings of the Nigerian Academy of Science (PNgAS)* is the peer-review official journal of the Nigerian Academy of Science, one of Africa's leading science Academies and the foremost independent scientific body in Nigeria. The regular edition of the journal is a multidisciplinary publication, with the primary objective of disseminating original research, systematic reviews, and meta-analysis in all Science, Technology, Engineering, and Mathematics (STEM) disciplines, especially those that address national and regional developmental challenges. The journal publishes articles that are based on deep-seated formative research using large and multi-center datasets that leads to a better understanding of the context of science-related developmental challenges and appropriate pathways for accomplishing change in the following scientific disciplines:

Physical Sciences	**Biological Sciences**
Mathematical Sciences Physics, Astronomy, and Space Sciences Chemical Sciences Engineering and Applied Sciences Earth and Environmental Sciences	Biochemistry, Molecular Biology, and Biotechnology Medical Sciences Pharmaceutical Sciences Biological Sciences Agricultural and Forestry Sciences Veterinary Sciences

The journal publishes one volume each year but would publish two to three volumes from 2020. The journal is primarily intended for use in the scientific community, but its multidisciplinary nature also makes it accessible to researchers, educators, students, and readers interested in current issues and development.

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An official journal of the Nigerian Academy of Science, the apex Science Society of Nigeria

1. The journal publishes articles emanating from outstanding original research.
2. From time to time, the Editorial board may request individuals to write commentaries on burning scientific issues.
3. Articles should be written in a language that is intelligible to most scientists.
4. Manuscripts for publication in the Proc. Nigerian Acad. Sci. should, preferably, be written or sponsored by a Fellow of the Academy. However, other authors are welcome to submit papers directly to the Editor-in-Chief. Authors may wish to suggest names of possible reviewers of the articles not from the same institution as the authors. The Editorial board makes final decisions on who may review an article.
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10. **Acknowledgements:** Funding sources and technical assistance are permitted but not dedications.

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EDITORIAL

Deepening interdisciplinarity in scientific research for impact and development

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Given the multi-faceted developmental challenges that Africa faces, it is not surprising that research that generates development and impact is now a regular discourse in the continent. While scientific research disciplines have grown considerably in recent times and now include domains such as genomics, proteomics, nanotechnology, nanomedicine, bioinformatics, and systems biology, it is evident that these will not translate to impact and development if inter-disciplinary research approach is not purposefully engaged.

The *Proceedings of the Nigerian Academy of Science* recognises inter-disciplinary approach to scientific research as a dominant strategy that can accelerate the pace of development in all sectors in the African region. Inter-disciplinary research also used interchangeably with "multi-disciplinary research', can be defined as the building of theory and scientific concepts at the interceptions between disciplines to enhance scientific validity and foster the attainment of impact (Choi and Pak, 2006).

While this type of research has always been known, its development has arisen by natural circumstances rather than by purposeful delivery. Indeed, a new paradigm called "trans-disciplinary research" is now being advanced to provide a systematic framework for moving scientific research into social, economic, political, and environmental spheres to influence human development and well-being (Choi and Pak, 2006).

In this edition of the *Proceedings*, we feature ten original research articles from various fields of science including physics, ecology, agriculture, chemistry, and mathematics, all designed to solve various specific problems in development. However, the extent to which the authors of the papers have used inter-disciplinary and multi-disciplinary approach to develop the research questions and to conduct the research remains a moot point. Although many frontline commentators and protagonists of science in the African region have often emphasised the importance of inter-disciplinary research (Ludwig *et al.*, 2011; Waldman, 2013; Rau *et al*.,

2018), it appears that this has tended to be rhetorical rather than practical. More and more, science researchers have tended to work in silos without consultation with their colleagues even in the same profession or departments, which reduces the quality of products and outcomes from such research efforts.

Worse still, science researchers have lacked the capacity or willingness to work with non-science researchers in disciplines such as law, sociology, economics, politics, anthropology, history, and the like. And yet, much of Africa's developmental challenges lie in the intersections between subjects such as history, culture and traditions, patriarchy and gender, and religion, with some of the most endearing principles of science. It is increasingly evident that science applications and solutions in Africa cannot work effectively without addressing these underpinning circumstances. Indeed, Europe and other more developed regions of the world may have risen above these traditional and primordial practices, but it is important to note that they began in their early days with those elements, and only by overcoming them did they discover the pathway to development.

As evidence of the importance of inter-disciplinary research to development, it must be remembered that many of the major developments that have taken place in the world would not have occurred without inter-disciplinary research. An example is the sequencing of the human genome which took place in the new millennium, which was a collaborative endeavour between different disciplines including physics, chemistry, biology, computer, mathematics, and bioinformatics (Hood and Rowen, 2013). Also, the discovery in 1946 that nucleic acid can be oriented in a magnetic field led to the development of the modern field of nuclear magnetic resonance and magnetic resonance imaging, a field that has been propelled by scientists working collaboratively in various disciplines, including biology, chemistry, physics, neuroscience, and psychology. To date, several Nobel prizes have been awarded for this seminal work, and indeed, we are not aware of any Nobel Prize that have so far been awarded for solo research efforts (Luiten, 1999).

Clearly, the need for inter-disciplinary research to propel development in the African region cannot be over-emphasized. However, the challenge we see is the inherent lack of capacity within Africa's research community to engage in inter-disciplinary research. Traditional training curricula have tended to address the needs of specific disciplines without provisions made for cross-boundary and cross-disciplinary interactions. This has led to science professionals who do not understand the needs of other disciplines, and who in some instances may antagonise those needs. Indeed, what we see as festering inter-professional rivalries today may be a manifestation of the lack of understanding of the needs and practices in adjoining disciplines.

A distinct example is the training provided for quantitative and numeric research in many science disciplines, with little commensurate training in qualitative research. By contrast, social science researchers tend to be better trained in qualitative research. Thus, in mixed methods research that seek a triangulation of qualitative and quantitative methods to generate better understanding of the social context of specific quantitative results and to plan interventions, there often tends to be complete dissonance in interactions between collaborating science and social science researchers. Indeed, this is one of the explanations for the paucity of inter-disciplinary research between science-based and social science researchers, a situation that curtails the quality of intervention and translational research emanating from the region. A new approach to curricula

development is to harmonize curricula to make specific provisions for trainees to cross boundaries in their understanding of complex problems. The new approach would require trainees and trainers not only to focus on their disciplines but to specifically target their training towards solving overall developmental challenges, which inevitably will require increasing knowledge and skills in other disciplines.

It is for this reason that the African Academy of Science has made provisions for non-science researchers especially in the social sciences to become fellows of its Academy. The African Academy also makes specific provisions for inter-disciplinary research and promotes the adoption of inter-disciplinary and multi-disciplinary principles in the research grants it provides for development in the region (AAS, 2020). We are hopeful that other Science Academies and indeed, all networks that promote research for development in all disciplines in the African region will adopt this approach as most needed for growth and development in the continent.

In conclusion, there can be no doubt that the solutions to complex developmental problems in all spheres of human life require experts and expertise in many different disciplines – and increasingly expertise in different fields that go beyond the pure sciences. Individuals must be broadly trained so that they can understand and contribute to research that overlaps different fields of development.

Declaration of Interest: None to declare

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COMMENTARY

Covid-19 preparedness and response: experiences of the Nigerian Institute for Medical Research

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SUMMARY

The global community is facing a health crisis caused by coronavirus disease 2019 (COVID-19). The coronavirus pandemic is severely disrupting the global economy. Countries are battling to slow the spread of the virus by testing, employing contact tracing, restricting travel, quarantining citizens, and encouraging use of face mask, hand hygiene and social distancing

measures. The lockdown imposed in many countries including Nigeria has resulted in increased cost and shortages of reagents and supplies worldwide. Due to the highly contagious nature of the disease, rapid rate of spread, and lack of an effective therapy, it became necessary for nations of the world to mount an efficient response mechanism to curb the spread of the pandemic. The Nigerian Institute of Medical Research (NIMR) has responded actively to the current pandemic with some innovations with respect to sample collection systems, molecular diagnostics, kit development and validation. Due to the highly infectious nature of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) the causative agent of COVID-19, the institute also invested in the production of infection control tools. The extent of response by the institute would not have been possible but for collaboration and partnership with well-meaning organizations and stakeholders. National, State and public cooperation are very essential for effective response to any pandemic. The response of NIMR to the pandemic is herein discussed. Lessons learned and recommendations made are also shared to help institutions interested in combating this and future pandemics of similar nature.

Key Words: *Pandemics, preparedness, COVID-19, response*

ISSUES

The global community is facing a health crisis caused by a pandemic due to coronavirus disease 2019 (COVID-19). Pandemics have been a part of human history but the most fatal recorded was the Black Death, also known as The Plague. It killed between 75-200 million people in the 14th century (1-3). Other major pandemics recorded include the Plague of Athens, which occurred between 430-426 BC. It occurred during the Paloponnesian war and claimed about two-thirds of the Athenian population (4). Since then, there have been several other pandemics globally, including the Antonine plague of 165 AD which killed five million (5), Cyprian plague of 251 AD killed 5,000 people a day in Rome and the Justinian plague of 541 AD claimed 10,000 lives a day (6) resulting in the death of about half the human population of the then world (7).

A pandemic is a sudden occurrence of an infectious disease with a worldwide geographic spread affecting a high proportion of the population with sustained person to person transmission. Additionally, it has a very high death toll with devastating effects because it is usually caused by a new organism against which the infected persons have no immunity. The Spanish flu of 1918-19 was one of the deadliest pandemics. It was reported to have killed at least twenty million people, more than twice the number killed in the military action in World War 1 (8). Nigeria also witnessed the Spanish Flu pandemic caused by Influenza virus, which claimed about 500,000 lives of the 18 million Nigerian population in less than six months (9). It spread fast and wide without much difficulty and, 50-80% were stricken by the virus. Some pandemics, which have occurred in 21st century, include the H1N1 pandemic of 2009 which caused no fewer than 300,000 deaths and the SARS pandemic of 2003 which infected 8,096 people from 27 countries and claimed 774 lives (10). Currently, the pandemic ravaging the world is COVID-19. This disease caused by SARS-CoV-2, originated from Wuhan, China in December 2019 and it has spread to 213 countries globally with 6,875,191 confirmed cases and 398,689 deaths as of June 6, 2020.Africa has confirmed 179,997 cases with 4,954 deaths while Nigeria, third most impacted in the continent confirmed 11,844 cases and 333 deaths as of June 6, 2020 (11).

Generally, pandemics spread rapidly around the world, posing enormous health, economic, environmental and social challenges to the entire human population. The coronavirus outbreak is severely disrupting the global economy. Countries are battling to slow the spread of the virus by testing and treating patients, employing contact tracing, restricting travel, quarantining citizens, and cancelling large gatherings such as religious meetings, sporting events, concerts and schools in other to ensure social distancing. The pandemic is spreading fast with more severe effects on the aged and persons with underlying health conditions. COVID-19 is much more than a health crisis; by stressing every one of the countries it touches, it has the potential to create devastating social, economic and political crises that could leave deep scars (12). Due to the severe impact that pandemics could have, it is important that health allied agencies respond effectively to mitigate their impact. The response to COVID-19 by the Nigerian Institute of Medical Research (NIMR) is herein discussed.

DESCRIPTION

Molecular detection and sequencing of SARS-CoV-2

NIMR has a mandate to research into communicable and non-communicable diseases of public health importance in the country. In her quest to accomplish this mandate, the institute collaborates with other institutions within and outside the country. It was such collaboration with China Center for Disease Control that paved way for two of our staff to attend a three-month training program in China on Molecular Diagnosis and Pathogen Identification Techniques for Emerging and Reemerging Infectious Diseases. The aim of this training was to improve laboratory capacities of the participants in conducting molecular detection assays and implementing molecular diagnosis for potential pathogens associated with emerging and reemerging infectious diseases. The training also had an objective of developing leadership in scaling up capacity in the home countries of participants. Our staff was away from July to September 2019 and upon return, they had acquired skills for:

- Development of fast point of care PCR panels for febrile illnesses
- Design of primers for prompt identification and diagnosis of arboviruses and other viruses of interest.
- Targeted and whole genome sequencing and designing of the primers needed for them.
- Discovery of viruses and other pathogens using next generation sequencing (NGS)
- In house production of enzyme linked immunosorbent assay (ELISA) kits

Similarly, three other staff were at the Institute Pasteur, Dakar, Senegal between September and October 2019 to acquire more skills on the use of NGS and detection of viruses using in-house serological assays. Upon their return from the training, a viral surveillance team was set up in November 2019 to harness the skills they had acquired in preparation for any viral outbreak in the country. The team met biweekly to set up plans, and design assays in preparation for any eventual viral outbreak. Purchase orders were placed; primers and probes were designed and purchased. One of the Chinese collaborators visited the institute in January 2020, at the onset of the coronavirus outbreak in Wuhan, China. It was then agreed that some qPCR kits be obtained for diagnosis of COVID-19 in Nigeria. At that time, we designed primers and probes specifically for conventional PCR and sequencing of beta coronavirus. By February 2020, some companies in-country requested to screen their Chinese workers returning from their lunar festival for SARS-CoV-2, before commencement of work. This service was provided with ease as we were prepared for it. The management of the institute formally informed the Honourable Minister of Health of Nigeria of our preparedness to respond to the pandemic in the country. As soon as the

index case was detected, an aliquot of the extracted RNA and a COVID-19 blind panel from Nigeria Center for Disease Control were sent to NIMR to confirm our laboratory readiness for the response. The samples were successfully tested at the Center for Human Virology and Genomics (CHVG) on February28, 2020 and we proceeded to sequence the index case using the Sanger sequencing technology (13). The institute has since deposited some partial and full-length genome sequences in the GenBank (Accession nos. MT159778; MT344135). Subsequently, CHVG was included in the network of COVID-19 testing laboratories in Nigeria.

In addition, the institute supports the Lagos State Biobank with testing of samples collected from their decentralized sample collection centers. CHVG has access to three qPCR machines and a COBAS 6800 (Roche) system, which together can generate 700 test results daily, if fully optimized. In addition, the Center for Tuberculosis Research (CTBR) in NIMR, currently has three GeneXpert machines, has been designated for COVID-19 testing in Lagos state. The CTBR is on standby for activation for COVID-19 testing any moment from now. As of June 6, we have tested >6,000 samples with results all issued out. Furthermore, CHVG has provided some back-up testing support to other laboratories in the COVID-19 network.

Establishment of the first drive-through sampling centre in Nigeria

The institute with the support of one of her partners, Life Bank, established a modified drive-through center (submitted for publication) where samples are collected daily for testing. Many persons register daily on our website (https://nimr.CoVID.com.ng/) to access COVID-19 diagnosis through our drive through center. However, we are unable to invite all of them to the facility because only about 150persons can be attended to daily. Consequently, we had to introduce another innovation to meet the demand using telemedicine approach in collaboration with Mobilhealth International. This is a self-sample collection model to increase access toCOVID-19 testing in the institute. This approach minimizes person to person contact presently being experienced at the modified drive through sample collection center.

COVID-19 research at NIMR

Development of point of care (POC) diagnostic assays

As testing services were being provided, research was also prioritized in line with our mandate. In anticipation of stock out of test kits due to the global demand for same reagents, we embarked on the development of in-house assays that can be deployed for field and laboratory use to control the pandemic. Primers and probes for detection of SARS-CoV-2 using qPCR were designed and are being evaluated and optimized. Earlier, NIMR had developed a DNA extraction kit. Subsequently, an RNA extraction kit suitable for COVID-19 was developed and is presently being validated.

The institute also adopted another approach for the development of rapid, sensitive, and specific nucleic acid testing platform for diagnosing SARS-CoV-2. This is an isothermal point-of-care (POC), field test systemdeveloped by our staff as part of skills acquired, during the training in China.This POC platform was utilized to develop sensitive multiplex PCR diagnostic assays for febrile illnesses. In six tests, these febrile illness assays differentially detect the top 10 causes of febrile illness in children, including malaria (*Plasmodium falciparum*), typhoid Fever (*Salmonella enterica* serovar *typhi* and *paratyphi*), *Streptococcus pneumoniae, Brucellaabortus, Leptospira interrogans*, Dengue 1-3 viruses, yellow fever virus, rotavirus A and chikungunya virus. These assays are presently being validated and field tested. Subsequently, this technology and platform is being used to develop multiplex qPCR assay for diagnosing SARS-CoV-2 and

Influenza A virus in one test. Currently, the assay primers and probes have been synthesized and received in the laboratory. Also, isothermal reagents and equipment for pilot testing have been purchased.

Other plans to develop rapid diagnostic tests (RDT), enzyme linked immunosorbent assays (ELISA) and candidate vaccines are still at the initial phase of development.

Evaluation of commercially available rapid diagnostic tests (RDTs)

Several RDTs and ELISA kits have flooded the country seeking for evaluation. The CHVG is a WHO prequalification evaluation laboratory with long experience in validation of molecular and serological test kits. Thus, RDTs and ELISA assays are being validated at the CHVG to determine their performance characteristics on behalf of the National Agency for Food and Drug Administration and Control. Results obtained guide in determining their approval for registration in the country. Several other COVID-19 related research projects embarked upon at NIMR include, amongst others, nationals ero-epidemiological survey, co-morbid infections associated with COVID-19and clinical trial of the safety and efficacy of chloroquine phosphate, hydroxychloroquine sulphate, Azithromycin, and lopinavir/ritonavir for treatment of COVID-19 in the country.

Production of infection control materials

The lockdown imposed in many countries of the world, with attendant economic stagnation has resulted in increased cost and shortages of reagents and supplies globally. It is said that "necessity is the mother of invention". As such, NIMR has looked inward to generate infection control materials required to combat the pandemic. We have within CHVG, developed a standard protocol and began production of hand sanitizers and decontamination solutions for the entire institute. As a matter of fact, a private company has entered into an agreement with the institute to supply the hand sanitizers commercially for public use. In the same manner, face masks produced in bulk by another company are sent to NIMR where they are sterilized using UV rays and packaged for public use. Prior to this pandemic, the institute had two tissue culture laboratories, which produced viral transport medium (VTM) to maintain cell lines. At the nick of time, when VTM became a scarce commodity in the country, the laboratories began commercial production of VTM for use in sample collection at the national and state levels as well as for some private organizations. All these ventures became some source of revenue for the institute. So, while the pandemic was taking its toll in the country, we were discovering our strengths for improved productivity.

Biosafety procedures at NIMR

At the onset of the pandemic, a risk assessment was conducted within the three centres in the microbiology department of the institute. A standardized template from WHO website was used and gaps identified were closed. All technical staff interested in the response undertook the WHO infection prevention control training for COVID-19 and their certificate of training was a requirement to enrol in the response. Additional trainings, such as doffing and donning of PPEs and preparation of decontaminants were also conducted. Non-technical staff such as storekeepers, security personnel, clerical staff on the team were also provided relevant trainings` to ensure their protection. A daily log for COVID-19 symptoms was deployed to all staff to monitor early signs of infection; this was to help early detection of COVID-19 infection and to prevent transmission amongst our staff. Some essential staff were lodged in a research suit within the institute to minimize contact with their families in order to prevent infection.

Furthermore, staff were encouraged to test at intervals when they had suggestive symptoms or before visiting their families.

We adopted the modified drive through centre because it is noted to protect health care workers from infection. A drive through sample collection centre has an advantage of reducing contact and exposure time, thus reducing risk of infection transmission to health care workers and clients (14). Even though WHO recommends Biosafety Level 2 (BSL-2) for non-propagative diagnostic laboratory work like sequencing and nucleic acid amplification test (15), since the institute has a biosafety level 3 (BSL3) laboratory, all samples received for testing are inactivated in the BSL3 laboratory before further processing in the BSL2 laboratory.

LESSONS LEARNED

Top leadership involvement and investment in training

There is a saying that "success is where preparation and opportunity meet". This is what describes our response. This explains why the institute invested in training her staff in foreign countries. The knowledge gained was also harnessed into productive use. When the opportunity came knocking, the institute was ready to respond. One major strength that facilitated the strategic and prompt response from the institute in combating the raging pandemic is the dynamic leadership style of the top management. The involvement of the top leadership was very apparent, solutions and suggestions were provided for challenges that came along the way. Staff were encouraged to think outside the box to break new grounds. Established protocols had to be circumvented in order to make progress. A response team was set up with personnel drawn from different departments within the institute and some volunteer staff were engaged. A reporting structure was put in place and the team went straight into action with review meetings held twice weekly. The commitment of the top management was quite motivating, and this reinforced teamwork among the staff. All staff worked hard and long in response to the pandemic. Some staff got infected in the course of work, were admitted in different isolation centers in Lagos, recovered, were discharged and have resumed work. Despite this experience, the staff were not deterred; they are still willing to lay down their lives to fight the pandemic. This is without any commitment to remuneration nor promise of hazard allowance. This uncommon gesture is worthy of emulation by other Health Care Workers.

Collaboration and partnership

The extent of response by the institute would not have been possible but for the collaboration and partnership with well-meaning organizations and stakeholders. It was the collaboration with the China Center for Disease Control to foster China Africa Public Health Cooperation that enabled our staff training. Similarly, in December 2018, the institute had signed a memorandum of understanding with the Institute Pasteur, Dakar, Senegal to foster south-south collaboration to birth new feats in the field of research in the west coast region. This was what led to the opportunity for the training of some of our staff in 2019. Other non-governmental organizations that partnered with the institute include LifeBank that supported the establishment of a drive through testing center, provision of personal protective equipment (PPE) and bore some other expenses. Co-creation Hub Nigeria maintained the COVID-19 testing link on our website. Mobihealth anchored the self-sample collection innovation with their online application. Other organizations that supported the response in NIMR include Nigerian LNG limited, which supported with purchase of some reagents, Flour Mills, Nigeria Plc and AL Samparda Pharma Nigeria Ltd donated PPEs and other consumables required for the response. The goodwill we enjoyed from the electronic and print media also helped to facilitate our response.

Effective response to future pandemics

COVID-19 is still here with us. Research is in progress for the development and evaluation of effective therapeutic agents for treatment and vaccines for prevention of COVID-19. Meanwhile, concerted efforts must be directed at increased testing, contact tracing, isolation and non-therapeutic measures to contain viral spread. While COVID-19 may be reduced or eliminated at some stage, another deadly pandemic is likely to occur again, although it will be difficult to predict exactly when the next one will be. As the years go by, pandemics will become more frequent because of increased and unprecedented rates of human travels and other interactions with nature. A multi-disciplinary approach to problem solving will be required for adequate preparation and effective response to future pandemics, including identification and evaluation of herbal products and their potential roles. The 21st century pandemic response approach will need to be multifaceted and integrated cutting across several subject areas and disciplines such as, governance and infrastructure, engagement and communication, social sciences including anthropology and human geography, emerging technologies (pathogen genomics, data science, and artificial intelligence). Other subject areas include research and development that go beyond epidemiology to include, diagnostics, therapeutics and vaccine development, one health that connects human health to animal health and the environment and, ethical considerations requiring consent and innovative and adaptive clinical trial designs (16). Surveillance that is systematic, simple and consistent will be required for data aggregation and trend monitoring. Data quality and dissemination plans are also crucial attributes in this process. Modelling for prediction and forecasting will be necessary as well as implementation research that are aimed at turning basic research findings into health-improving products.

RECOMMENDATIONS

National, State and public cooperation are very essential for effective response to any pandemic. Because pandemic is a global problem, health diplomacy and collaboration with major global health institutions such as the World Health Organization is also crucial. Enhancing trust and cooperation, between people, regions, states and the federal government is paramount for a sustained fight against the pandemic (11).It is therefore important to work in collaboration with both the states and federal government in combatting the pandemic. The Nigeria Center for Disease Control supplied some kits, reagents and consumables required for testing while Lagos State Ministry of Health posted surveillance officers to the institute to work with the team. They completed case investigation forms for each client that visited and uploaded our results to the state and national database. They ensured infected persons from our testing center were evacuated and admitted in the isolation centers. Therefore, in conducting the COVID-19 testing, it is essential to collaborate with the appropriate authorities.

However, it is also essential that staff remuneration and hazard allowances are settled by the appropriate authorities as this will help sustain enthusiasm of the staff. This is especially important as it relates to a highly infectious agent as SARS-CoV-2, which is infecting health workers daily despite their boldness in combatting the pandemic. Infection control measures need to be at its maximum in combating this and other pandemics (17, 18). It is essential that adequate personal protective equipment (PPE) is provided for frontline staff, and training on infection prevention and control is very essential. Preventing staff burn out is also very critical, As work intensity and inadequate rest may make them prone to infection (19). The importance of these measures cannot be overemphasized.

Since the COVID-19 outbreak, the global supply chain for PPE and relevant test kits and reagents has not adequately functioned to meet the surge in demand. Constraints in supply and logistics, including export bans for PPE and key materials, have become an unpleasant reality (20). It is, therefore, important to encourage indigenous production of PPE materials, kits and supplies for national use. The government and private sector should invest in purchase of essential equipment like the oligo synthesizers to facilitate local production of relevant primers, probes and kits for diagnostic use in the country, and automated sample nucleic acid extraction systems. Grants should be awarded for such production ventures and the outcome of such will indeed lead to research and development for national growth. This pandemic thus provides an opportunity for national development and this opportunity should be fully utilized to the benefit of the country because future pandemics are bound to be more frequent than they have ever been.

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ORIGINAL RESEARCH ARTICLE

Antibiogram of bacterial flora of public health significance associated with postharvest *Irvingia gabonensis* seeds in Lagos State, Nigeria

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Abstract

The spread of resistant bacteria within the community has continually posed obvious additional problems for infectious control. Efforts to identifying sources of resistant bacteria have not been channeled towards medicinal food condiments. This study investigated the antibiogram of bacterial flora of public health significance associated with postharvest *Irvingia gabonensis* seeds in Lagos State, Nigeria. The blended, homogenized and serially diluted samples of *I. gabonensis* seeds were plated using the spread plate technique on selective and differential agar media. API 20E and API 20NE were used for identification of members of *Enterobacter*iaceae and non-*Enterobacter*i*aceae*, respectively. The agar disc-diffusion method was employed to determine the antibiotic resistance profiles of the isolates. A total of 263 bacterial isolates (129 Gram-positive and 134 Gram-negative) were encountered. Eighty-five (66%) of Gram-positive isolates exhibited resistance to penicillin, gentamicin 65 (50.4%), erythromycin 69 (53.5%), cloxacillin 63 (43.8%), chloramphenicol 73 (56.6%), amoxicillin 75 (58.1%), tetracycline 58 (45%) while 69 (53.5%) showed resistance to streptomycin. However, 87 (65%) of Gram-negative bacterial strains exhibited resistance to cloxacillin, ceftazidime 82 (61.2%), ciprofloxacin 67 (50%), gentamicin 77 (57.5%), cefotaxime 74 (55.2%), augmentin 84 (62.7%), nitrofuratoin 61 (45.5%) and 24 (17.9%) to ofloxacin. This study showed that *I. gabonensis* seeds could be a source of antibiotic-resistant bacterial strains, despite its enormous medicinal properties, which is a threat to public health. The antibiotic resistance patterns of isolated bacterial strains are of medical importance as there are chances of transferring resistant traits.

Keywords: *Antibiogram, antibiotic resistance, bacteria, Irvingia gabonensis, public health.*

INTRODUCTION

Irvingia is a genus of African and Southeast Asian trees in the family Irvingiaceae. It is considered as one of the most domestically consumed wild fruit tree as it is a dominant tropical forest tree of Central and West Africa. In Nigeria, particularly in the southern and Eastern regions, *Irvingia gabonensis*, also known as bush mango, African mango seed, dika nut or apon, is grown and consumed basically because of its kernels which serve as a major condiment in the preparation of Nigeria's famous "*ogbono*" or "*apon*" soup.[1]

They bear edible mango-like fruits (Plate 1), and are especially valued for their fat and protein-rich nuts.[2] The subtly aromatic nuts (Plate 2) are usually sun-dried in order to preserve, and could be sold in the form of powder or as whole. Dika bread or Gabon chocolate is produced from the paste form of *Irvingia* seeds. Their high content of mucilage enables them to be used as thickening agents for dishes such as ogbono or *apon* soup. Vegetable oil could also be extracted from these seeds. The fruit is a large drupe, with fibrous flesh.

Adegbehingbe *et al*.[2] reported that the seeds of legumes account for up to 80% of dietary protein and could be the only source of protein for certain group. The cooked forms of the seeds could be eaten as meals while the fermented forms as condiments that enhance the flavors and texture of foods. The condiments could serve as a delicious complement to sauces or soups to substitute for meat or fish. Fermented legumes have characteristic organoleptic properties, which probably are the most important factors attracting consumers.[3]

Studies have shown that seed extract of *I. gabonensis* caused a significant reduction in body weight among obese people.[4] Earlier studies had attributed a reduction in the incidence of diseases such as cancer, cardiovascular disease, cataracts, and brain and immune dysfunction to consumption of fruits and vegetables which in turn was credited to natural antioxidant phytochemicals inherent in them.[5]Ekpe *et al*.[6] also showed that *Irvingia* seeds had high antioxidant capacity.

The soluble fibre of the seed of *I. gabonensis* improves gradual absorption of dietary sugar as it has the potentials to delay stomach emptying. This helps reduce the elevation of blood sugar levels that is typical after a meal. The fibre of *I. gabonensis* seed has the potentials to bind to the gut bile acids and remove them from the body, so that the body could convert more cholesterol into bile acids. Several grams per day of *I. gabonensis* may help reduce total blood cholesterol, low-density lipoprotein (LDL) cholesterol, and triglycerides and in some cases raise high-density lipoprotein (HDL) cholesterol.[7] *I. gabonensis*, however, has the tendency to convey potential pathogens from farm to kitchen.

Plate 1: *Irvingia gabonensis* whole fruit

Plate 2: Dried seeds of *Irvingia gabonensis*

Some bacteria have the ability of developing resistance to antibiotics. Antibiotics are placed into different categories based on their major mechanisms of action which include interference with protein synthesis (for instance, tetracyclines and macrolides), inhibition of cell wall synthesis (β-lactams and glycopeptide agents), disruption of bacterial membrane structure (daptomycin and polymyxins) and inhibition of nucleic acid synthesis (rifampin and fluoroquinolones), metabolic pathway inhibition (trimethoprim-sulfamethoxazole).[8] Bacteria may exhibit intrinsic resistance

to one class of antibiotics, while acquisition of resistance genes from other organisms or *de novo* mutation, is also possible.

Bacterial resistance often results in treatment failure, which can have serious consequences, especially in critically ill patients.[9] Acquired resistance genes may enable a bacterium to produce enzymes that destroy the antibacterial drug. There could be expression of efflux systems to prevent the drug from accessing its intracellular target, which could modify the target site of the drug, or create an alternative metabolic pathway that bypasses the action of the drug. Transformation, conjugation, or transduction could lead to the acquisition of new genetic material by antimicrobial-susceptible bacteria from resistant strains of bacteria, with transposons often mediating the introduction of multiple resistance genes into the host's genome or plasmids. Use of antibacterial agents creates selective pressure for the emergence of resistant strains.[10]

Prolonged therapy with certain antibiotics, such as linezolid or vancomycin, could as well lead to the development of low-level resistance which compromises therapy.[11] The spread of resistant bacteria could become broader infection-control problems within healthcare institutions and communities. Medically important bacteria are increasingly observed in the community.[12,13,14] Obvious additional problems for infection control are as a result of the spread of resistant bacteria and which are rarely traced to some of these food condiments. This work aims at evaluating the antibiogram of bacterial flora of public health significance associated with postharvest *Irvingia gabonensis* seeds.

MATERIALS AND METHODS

Sources of Samples

Irvingia gabonensis seeds were purchased from local sellers from five markets in Lagos State, Nigeria. Samples from twenty-five different locations were separately blended into powdery form using an electric blender (Model 242 NAKAI, JAPAN). These were kept in sterile polythene bags in the refrigerator at 4 ^{o}C and then transported to the laboratory in sterile bags packed in insulated containers with ice packs.

Bacteriological Analyses

Bacteriological analyses were carried out in the Microbial Biotechnology Unit in the Department of Biological Sciences, North-West University, South Africa. Ten grams of ground *I. gabonensis* seeds were added to 90 ml of 0.1% (W/V) sterilized peptone water in a beaker and allowed to stand for 5 mins with occasional stirring. The homogenized samples were serially diluted to up to 10^{-8} and plated in duplicates on respective media using the spread plate technique. All plates were incubated at 32 °C for 24 - 48 hours. The plate count agar (PCA) was employed to enumerate aerobic mesophilic bacteria (AMB) while violet, red bile agar (VRBA) was used for the cultivation of coliforms. Colonies showing purple red colouration surrounded by reddish zone of precipitated bile were considered as coliforms. Enterobacteriaceae were counted on MacConkey agar, and pink to red purple colonies with or without haloes of precipitation were regarded as member of Enterobacteriaceae. *Streptococcus* spp were isolated on blood agar plates and characteristic colonies showing β-haemolysis were confirmed as streptococci. *Bacillus* spp were cultivated on mannitol/eggs yolk/polymyxin agar (MYP). All motile, catalase and Voges Proskauer positive isolates with ellipsoidal spores were confirmed as *B. cereus*. *Staphylococcus aureus* were cultivated on mannitol salt agar (MSA).

Characterization and identification of isolates

Representative colonies were picked and further purified by repeated subculturing on PCA. Pure cultures were preserved on nutrient agar slants at refrigeration temperature of 4 °C. Cell morphology, Gram's reaction, colony characterization and biochemical characterizations of isolates were performed according to standard procedures.[16,17] API 20E and API 20NE were used for additional identification of members of *Enterobacteriaceae* and non-*Enterobacteriaceae*, respectively.

Antibiotic susceptibility of isolates

The bacterial isolates were investigated for their susceptibilities to various antibiotics using the agar disc diffusion as described by Clinical and Laboratory Standard Institute Guidelines[18]. Antibiotics discs used were penicillin (PEN; 25µg), chloramphenicol (CHL; 30µg), gentamicin (GEN; 10µg), cloxacillin (CXC; 30µg), ampicillin (AMP 30µg), erythromycin (ERY; 5µg), streptomycin (STR; 10µg) and tetracycline (TET; 30µg) for Gram-positive bacteria. For Gram-negative bacteria, cloxacillin (CXC; 30µg), ceftazidime (CAZ; 30mg), ciprofloxacin (CRX; 10µg), gentamicin (GEN; 10µg), cefotaxime (CTX; 30µg), augmentin (AUG; 30µg), nitrofurantoin (NIT; 250µg) and ofloxacin (OFL; 30µg) were investigated. The entire surface of each sterile Mueller-Hinton agar plate was inoculated with 500 µl of each bacterial isolate, using sterile swab sticks. The plates were left for about 15 minutes before aseptically placing the antibiotic discs on the agar surfaces with sterile forceps, followed by incubation at 35 ^{o}C for 18-24 hours. The diameters of zones of inhibition were measured and recorded in millimetre, while zones of inhibition less than 10.0 mm in diameter or absence of zones of inhibition were recorded as resistant or negative.

RESULTS

Table I shows the morphological and biochemical characteristics of bacteria isolated from postharvest *I. gabonensis* seeds. A total of 263 bacteria were isolated from samples with 129 Gram-positive and 134 Gram-negative isolates. These were characterized into twelve bacterial species. The Gram-positive isolates were identified as *Bacillus cereus*, *Bacillus subtilis*, *Micrococcus luteus*, *Staphylococcus aureus*, *Staphylococcus epidermidis*, and *Streptococcus pyogenes* while the Gram-negative bacteria were *Enterobacter aerogenes*, *Escherichia coli*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, *Proteus mirabilis* and *Serratia rubidaea. P. aeruginosa* had the highest percentage occurrence of 14.07%, followed by *S. aureus* (12.17%), *S. epidermidis* (9.51%), *B. subtilis* (9.13%), *K. pneumoniae* (8.75%), *P. mirabilis* (7.99%), *S. rubidaea* (7.61%), *E. coli* (6.46%), *M. luteus* (6.46%), *E. aerogenes* (6.08%), *B. cereus* (6.08%) while the lowest was *S. pyogenes* with the percentage occurrence of 5.7%.

Table II shows the antibiotic resistance profile of Gram-positive bacterial isolates from postharvest *I. gabonensis* seeds in Lagos, Nigeria. Out of 129 Gram-positive bacterial isolates, 85 (66%) exhibited resistance to penicillin, gentamicin 65 (50.4%), erythromycin 69 (53.5%), cloxacillin 63 (43.8%), chloramphenicol 73 (56.6%), amoxicillin 75 (58.1%), tetracycline 58 (45%) while 69 (53.5%) showed resistance to streptomycin. *B. cereus* exhibited percentage resistance ranging from 31 to 68.8% as encountered in tetracycline and penicillin, respectively. The percentage resistances of *B. subtilis*, *M. luteus*, *S. epidermidis*, *S. aureus* and *Streptococcus pyogenes* ranged from 37.5 - 70.8%, 29.4 - 100%, 40 – 60%, 37.5 – 62.6% and 46.7 – 86.7%, respectively. The highest percentage of resistance was shown to penicillin by the Gram-positive bacterial isolates while the lowest was shown to tetracycline.

Similarly, from 134 Gram-negative bacterial strains, 87 (65%) were resistant to cloxacillin, ceftazidime 82 (61.2%), ciprofloxacin 67 (50%), gentamicin 77 (57.5%), cefotaxime 74 (55.2%), augmentin 84 (62.7%), nitrofuratoin 61 (45.5%) and 24 (17.9%) to ofloxacin. *E. aerogenes*, *E. coli* and *P. mirabilis* strains showed percentage resistances ranging from 0 – 100% while *K. pneumoniae*, *P. aeruginosa* and *S. rubidaea* strains exhibited percentage resistances ranging from 26.1 - 73.9%, 29.8 – 78.4% and 0 – 75%, respectively. Gram-negative bacterial strains exhibited highest level of resistance to cloxacillin and the lowest to ofloxacin (Table III).

Table I: Morphological and biochemical characteristics of bacteria isolated from postharvest *I. gabonensis* seeds in Lagos, Nigeria

Gram Reaction	Cellular morphology	Catalase	Oxidase	Coagulase	Indole	Motility	Methyl-Red	Voges Proskauer	Urease Activity	Citrate Utilization	Starch Hydrolysis	Gelatin Hydrolysis	Casein Hydrolysis	Spore Test	NO_3 Reduction	Glucose	Sucrose	Arabinose	Maltose	Mannitol	Xylose	Galactose	Sorbitol	Invositol	Raffinose	Frauction	No. of Isolates	% Occurrence	Most Probable Identity
+ve	R	+	+	-	-	+	-	+	-	+	-	+	-	+	-	+	+	-	-	+	-	-	-	-	+	+	16	6.08	*B. cereus*
+ve	R	+	+	-	-	+	-	+	+	+	-	+	-	+	-	+	+	-	-	+	-	-	-	-	-	+	24	9.13	*B. subtilis*
+ve	C	+	+	+	-	-	-	-	±	-	-	+	-	-	-	+	+	-	+	+	-	-	-	-	ND	-	17	6.46	*M. luteus*
+ve	C	+	-	+	-	-	-	+	+	-	-	+	+	-	+	+	+	-	+	+	-	+	ND	ND	ND	+	32	12.17	*S. aureus*
+ve	C	+	-	-	-	-	-	+	+	-	-	+	-	-	-	+	+	-	-	-	-	-	ND	ND	ND	+	25	9.51	*S. epidermidis*
+ve	C	-	-	-	-	-	-	-	-	-	-			-		+	+	-	+	-	-	+	-	ND	-	ND	15	5.7	*S. pyogenes*
-ve	R	+	-	-	-	+	-	+	-	+	+	-	-	-	+	+	+	+	+	+	+	+	+	+	ND	+	16	6.08	*E. aerogenes*
-ve	R	-	+	-	-	+	+	+		+	-	-	+	+	+	+	+	+	-	+	+	-	-	-	-	+	17	6.46	*E. coli*
-ve	R	-	-	-	-	-	+	+	+	+	+	-	-	-	-	+	+	+	+	+	-	+	-	+	-	-	23	8.75	*K. pneumoniae*
-ve	R	+	+	-	-	+	-	+	-	+	-	+	-	-	+	+	+	+	+	+	+	+	-	-	+	+	37	14.07	*P. aeruginosa*
-ve	R	+	-	-	-	+	+	-	+	+	-	+	-	-	+	+	-	-	-	-	+	+	-	-	-	ND	21	7.99	*P. mirabilis*
-ve	R	+	+	-	-	+	-	+	-	+	+	+	-	-	-	+	+	+	+	+	-	-	-	-	+	+	20	7.61	*S. rubidaea*

Keys: R = Rods; + = Positive reaction; - = Negative reaction; ND = Not determined; A = Zone A; B = Zone B; C =Zone C; D = Zone D and CT = Control.

Table II: Percentage antibiotic resistance of Gram-positive bacteria from postharvest *I. gabonensis* seeds in Lagos, Nigeria

Bacterial species	N	Antibiotics (µg/l)							
		PEN	**GEN**	**ERY**	**CXC**	**CHL**	**AMX**	**TET**	**STR**
B. cereus	16	11 68.8%	9 56.3%	9 56.3%	7 43.8%	10 62.5%	9 56.3%	5 31.3%	7 43.8%
B. subtilis	24	13 54.2%	14 53.3%	11 45.8%	9 37.5%	13 54.2%	15 62.5%	12 50%	17 70.8%
M. luteus	17	17 100%	8 47.1%	8 47.1%	9 52.9%	12 70.6%	15 88.2%	5 29.4%	10 58.8%
S. epidermidis	25	12 48%	10 40%	15 60%	12 48%	15 60%	12 48%	14 56%	13 52%
S. aureus	32	21 62.6%	15 46.9%	13 40.6%	19 59.4%	12 37.5%	17 53.15	15 46.9%	13 40.6%
S.pyogenes	15	11 73.3%	9 60%	13 86.7%	7 46.7%	11 73.3%	7 46.7%	7 46.7%	9 60%
TOTAL	129	85 66%	65 50.4%	69 53.5%	63 43.8%	73 56.6%	75 58.1%	58 45%	69 53.5%

Keys: N = Number of isolated strains; PEN = penicillin; GEN = gentamicin; ERY = erythromycin; CXC = cloxacillin; CHL = chloramphenicol; AMX = amoxycillin; TET = tetracycline; STR = streptomycin

Table III: Antibiotic resistance profiles of Gram-negative bacterial isolates from postharvest *I. gabonensis* seeds in Lagos, Nigeria.

Bacterial species	N	Antibiotics (µg/l)							
		CXC	**CXZ**	**CRX**	**GEN**	**CTX**	**AUG**	**NIT**	**OFL**
E. aerogenes	16	4 25.0%	0 0.0%	0 0.0%	8 50.0%	16 100%	16 100%	0 0.0%	0 0.0%
E. coli	17	9 52.9%	12 70.6%	9 52.9%%	17 100%	10 58.8%	7 41.2%	12 70.6%	0 0.0%
K. pneumonia	23	15 65.2%	17 73.9%	15 65.2%	6 26.1%	14 60.9%	13 56.5%	11 47.8%	11 47.8%
P. aeruginosa	37	29 78.4%	17 46%	11 29.8%	21 56.8%	19 51.4%	30 81.1%	20 54.1%	13 35.2%
P. mirabilis	21	21 100%	9 42.9%	15 71.4%	13 61.9%	8 38.1%	12 57.1%	7 33.3%	0 0.0%
S.rubidaea	20	5 25.0%	15 75.0%	10 50.0%	5 25.0%	5 25.0%	5 25.0%	5 25.0%	0 0.0%
TOTAL	134	87 65%	82 61.2%	67 50%	77 57.5%	74 55.2%	84 62.7%	61 45.5%	24 17.9%

Keys: CXC = cloxacillin; CAZ = ceftazidime; CRX = ciprofloxacin; GEN = gentamicin; CTX = cefotaxime; AUG = augmentin; NIT = nitrofurantoin; OFL = ofloxacin

The multiple antibiotic resistance (MAR) profiles of Gram-positive bacterial species from postharvest *I. gabonensis* seeds is shown in Table IV. The %MAR ranged from 25.0% to 87.5%.

The percentage multiple antibiotic resistances of the Gram-negative isolates from *I. gabonensis* ranged from 25% to 75.0% (Table V). A strain of *Serratia* species showed lowest %MAR of 25% while a strain of *P. aeruginosa* and three strains of *K. pneumoniae* exhibited the highest %MAR of 75.0%.

Table IV: Antibiotic resistance profiles of Gram-positive bacterial species from postharvest *I. gabonensis* seeds in Lagos, Nigeria

Bacterial species	No. of Strains	Antibiotics									
		PEN	GEN	ERY	CXC	CHL	AMX	TET	STR	MAR	%MAR
B. cereus	4				CXC	CHL	AMX			3	37.5
B. cereus	5					CHL	AMX	TET	STR	4	50.0
B. cereus	3				CXC	CHL			STR	3	37.5
B. cereus	2	PEN	GEN				AMX			3	37.5
B. cereus	2	PEN	GEN		CXC		AMX	TET	STR	6	75.0
B. subtilis	3	PEN		ERY	CXC		AMX	TET		5	62.5
B. subtilis	5	PEN		ERY	CXC		AMX		STR	5	62.5
B. subtilis	8	PEN	GEN	ERY	CXC	CHL			STR	6	75.0
B. subtilis	5	PEN	GEN	ERY	CXC	CHL	AMX			6	75.0
B. subtilis	3	PEN	GEN	ERY	CXC	CHL	AMX	TET		7	87.5
M. luteus	3		GEN	ERY		CHL	AMX		STR	5	62.5
M. luteus	2	PEN	GEN	ERY	CXC	CHL		TET	STR	7	87.5
M. luteus	3	PEN	GEN	ERY	CXC		AMX			5	62.5
M. luteus	9					CHL	AMX	TET		3	37.5
S. aureus	8	PEN	GEN		CXC		AMX	TET	STR	6	75.0
S. aureus	4		GEN	ERY	CXC		AMX	TET		5	62.5
S. aureus	2		GEN	ERY	CXC		AMX		STR	5	62.5
S. aureus	7		GEN	ERY	CXC	CHL	AMX			5	62.5
S. aureus	5					CHL	AMX	TET		3	37.5
S. aureus	9	PEN	GEN		CXC		AMX	TET	STR	6	75.0
S. epidermidis	6		GEN	ERY	CXC		AMX	TET		5	62.5
S. epidermidis	3		GEN	ERY	CXC		AMX		STR	5	62.5
S. epidermidis	8				CXC				STR	2	25.0
S. epidermidis	5		GEN	ERY	CXC	CHL	AMX			5	62.5
S. epidermidis	3					CHL	AMX	TET		3	37.5
S. pyogenes	2			ERY		CHL	AMX	TET	STR	5	62.5
S. pyogenes	1	PEN	GEN	ERY	CXC		AMX	TET	STR	7	87.5
S. pyogenes	5					CHL	AMX		STR	3	37.5
S. pyogenes	4				CXC	CHL	AMX	TET	STR	5	62.5
S. pyogenes	3				CXC	CHL	AMX			3	37.5

Keys: PEN = penicillin; GEN = gentamicin, ERY = erythromycin; CXC = cloxacillin; CHL = chloramphenicol, AMX = amoxicillin; TET = tetracycline; STR = streptomycin; MAR = Multiple antibiotic resistance; %MAR = Percentage multiple antibiotic resistance

Table V: Antibiotic resistance profiles of Gram-negative bacterial isolates from *I. gabonensis* seeds in Lagos, Nigeria

Bacterial Strains	No. of Strains	Antibiotics								MAR	%MAR
		CXC	CXZ	CRX	GEN	CTX	AUG	NIT	OFL		
E. aerogenes	7				GEN	CTX	AUG			3	37.5
E. aerogenes	6		CXZ			CTX	AUG			3	37.5
E. aerogenes	1	CXC				CTX	AUG	NIT		4	50.0
E. aerogenes	2				GEN	CTX	AUG			3	37.5
E. coli	4	CXC	CXZ	CRX		CTX		NIT		5	62.5
E. coli	7	CXC	CXZ	CRX		CTX		NIT		5	62.5
E. coli	3	CXC	CXZ	CRX	GEN	CTX				5	62.5
E. coli	3	CXC	CXZ		GEN	CTX		NIT		5	62.5
E. coli	1	CXC	CXZ	CRX	GEN	CTX		NIT		6	75.0
K. pneumoniae	5	CXC	CXZ				AUG			3	37.5
K. pneumoniae	4	CXC	CXZ	CRX			AUG	NIT		5	62.5
K. pneumoniae	2	CXC	CXZ	CRX	GEN	CTX		NIT		6	75.0
K. pneumoniae	6	CXC	CXZ	CRX			AUG			4	50.0
K. pneumoniae	3	CXC	CXZ	CRX		CTX		NIT		4	50.0
K. pneumoniae	3	CXC	CXZ	CRX	GEN	CTX				4	50.0
P. aeruginosa	6			CRX		CTX	AUG	NIT	OFL	5	62.5
P. aeruginosa	5			CRX	GEN	CTX	AUG	NIT	OFL	6	75.0
P. aeruginosa	2	CXC		CRX		CTX	AUG		OFL	5	62.5
P. aeruginosa	6		CXZ		GEN	CTX				3	37.5
P. aeruginosa	7		CXZ	CRX	GEN			NIT		4	50.0
P. aeruginosa	5		CXZ	CRX	GEN	CTX				4	50.0
P. aeruginosa	6		CXZ	CRX	GEN	CTX				4	50.0
P. mirabilis	4	CXC				CTX	AUG			3	37.5
P. mirabilis	5	CXC				CTX		NIT		3	37.5
P. mirabilis	5	CXC					AUG	NIT		3	37.5
P. mirabilis	3	CXC		CRX		CTX				3	37.5
P. mirabilis	4	CXC		CRX		CTX				3	37.5
S. rubidaea	7	CXC				CTX	AUG			3	37.5
S. rubidaea	5	CXC						NIT		2	25.0
S. rubidaea	4	CXC				CTX	AUG	NIT		4	50.0
S. rubidaea	4	CXC		CRX		CTX	AUG			4	50.0

Keys: CXC = cloxacillin; CAZ = ceftazidime; CRX = ciprofloxacin; GEN = gentamicin; CTX = cefotaxime; AUG = augmentin; NIT = nitrofurantoin; OFL = ofloxacin; MAR = Multiple antibiotic resistance; %MAR = Percentage multiple antibiotic resistance

DISCUSSION

Multiple antibiotic resistances were recorded among the bacterial flora associated with *I. gabonensis* seeds. It was noted that strains of same bacterial species exhibited varying susceptibility patterns to antibiotics. There was no single bacterial species whose strains were solely resistant or susceptible to one antibiotic. This is the basis for the importance of the roles of combined therapy. The fact that high multiple antibiotic resistance was recorded against most of the commonly used antibiotics in this study is a reflection of high prevalence of multiple antibiotic resistant bacteria in the community.

Despite the enormous health benefits associated with the consumption of *I. gabonensis* seeds as reported by several authors,[19-24] the presence of multidrug-resistant bacterial strains in the food

condiment is of public health concern. The isolation of potential pathogens in this study could be attributed to poor hygienic practices during harvest, transportation and/or storage of the seeds. The organisms encountered in this study have medical implications. For instance, *S. aureus* in food products is employed generally as a sanitation index. This indicates that the postharvest plant seeds had been exposed to conditions that might introduce or allow proliferation of pathogenic microorganisms.[14] *S. aureus* is an important food-poisoning organism because of its cosmopolitan distribution in nature and could be traced back to the environment as they are important normal flora of humans.[13]

S. epidermidis is the most frequently connected species from human epithelia. It is largely present on the axillae, head, and nares. The bacterium belongs to the group of coagulase-negative staphylococci (CoNS), which is distinguished from coagulase-positive staphylococci such as *S. aureus* by lacking the enzyme coagulase. The species displays a high degree of diversity with 74 identified sequence types. Among CoNS, *S. epidermidis* causes the greatest number of infections.[25] The bacterium represents the most frequent causative agent associated with infections of any type of indwelling medical devices, including peripheral or central intravenous catheters (CVCs).[26]

The presence of *B. cereus* in the food product could be attributed to the fact that the organism is usually found in soil, on decaying organic matter, vegetables and fomites, fresh and marine waters, and the intestinal tract of invertebrates, through which food products may become contaminated, and this leads to the transient colonization of the human intestine.[27] This organism is also frequently present in food production environments due to the adhesive nature of its endospores, which enables it to spread to all kinds of food.[28]

B. subtilis possesses the ability to transform itself into a spore and, thus, enters a dormant state, which allows it to withstand extreme environmental conditions. The bacterium holds the record for surviving in space for the longest duration, 6 years on a NASA satellite. *B. subtilis* is usually considered non-pathogenic. However, it has been implicated in food poisoning caused by poor quality bakery products among others. *B. subtilis* food poisoning has a rapid onset, with acute vomiting, commonly followed by diarrhoea.[29]

The contamination of the food product with *M. luteus* could be through soil, dust, water, or human skin. This bacterium can withstand huge doses of UV radiation and has the ability to degrade pollutants such as petrol. *M. luteus* played a crucial role in Fleming's discovery of lysozyme. It possesses the ability to exhibit dormancy without forming spores. *M. luteus* causes odours in humans during the process of breaking down the components of sweat.

The reservoir for *S. pyogenes* infection is humans and through which the food product must have got contaminated. Asymptomatic colonisations of the pharynx are found in up to 20% of the population during winter. Streptococcal pharyngitis is transmissible mainly through droplets, and occasionally through contaminated food and water. *S. pyogenes* can cause skin and soft tissue infections which usually can affect the muscles, the deeper tissue layers, and fascia. Toxin-mediated diseases are scarlet fever and the streptococcal toxic shock syndrome.[30]

E. aerogenes are generally distributed in water, soil, sewage, dairy products and vegetables. It is part of the commensal enteric flora and usually not pathogenic. However, some strains produce

shiga-like toxin. The bacterium has also been associated with nosocomial infections and a variety of opportunistic infections involving the urinary and respiratory tracts, and cutaneous wounds. *Enterobacter* spp are resistant to most antibiotics, especially the cephalosporins. Their resistance to β-lactam antibiotics, chloramphenicol, quinolones and tetracyclines have been well documented.[31,32]

The occurrence of *P. aeruginosa* in the food product could be attributed to its ubiquity. It is a common opportunistic pathogen in humans, causing a broad range of infections in community and healthcare settings.[33] The most serious manifestations of infection include bacteraemia (particularly in neutropenic patients), pneumonia (particularly in cystic fibrosis patients and critically ill patients), urinary tract infections and wound infections.[34] This organism is intrinsically resistant to many antibiotics and in recent years resistance has emerged to what were previously antimicrobial agents of choice.[35]

P. mirabilis is most commonly found in the human intestinal tract as part of normal human intestinal flora, alongside *Klebsiella* species and *E. coli*.[36] *P. mirabilis* is present in multiple environmental habitats, including hospitals and long-term care facilities. *P. mirabilis* causes 90% of *Proteus* infections and can be considered a community-acquired infection.[36]

E. coli is well recognized as the main commensal inhabitant of mammals' gastrointestinal tract. Pathogenic *E. coli* strains cause a number of human diarrhoea. These strains are serotyped on the basis of their O (somatic), H (flagellar), and K (capsular) surface antigen profiles into six categories: Enteroaggregative (EAEC), (EHEC)/Shiga toxin-producing *E.coli* (STEC), Enteroinvasive (EIEC), Enteropathogenic (EPEC), Enterotoxigenic (ETEC), and diffuse adherent (DAEC). EHEC is a subgroup of VTEC/STEC associated with 45 human diseases which, in addition to the verocytotoxin/shigatoxin producing capacity, harbors additional genes that are important in virulence. Verocytotoxin-producing *E.coli* (VTEC) is a term used to describe strains of *E.coli* characterized by the ability to produce verocytotoxin(s) (VT), or just verotoxins that are capable of killing Vero cells, a tissue culture line of monkey kidney cells.[37] Infections associated with these pathotypes are of public health concern.

The genus *Serratia* is a member of the broad Enterobacteriaceae family and has been differentiated into 10 species.[38] Infections of humans with *Serratia* are not as common as with more virulent members of the Enterobacteriaceae. *S. rubidaea*, which is a less well-described member of the genus, is majorly found in soil, water and food. The isolation of this species from food samples and clinical specimens is rare, but it may cause opportunistic infections in severely ill patients receiving broad-spectrum antimicrobials, or those that have undergone surgery or other invasive procedures.[39,40] When identified in clinical specimens, *S. rubidaea* is largely isolated from respiratory tract samples, skin wounds, faeces and bile.[41]

The increased level of resistance to antimicrobial drugs is a reflection of the indiscrimate use of antibiotics which is becoming a common practice without legal cautions in our environment.

CONCLUSION

This study showed that *I. gabonensis* seeds could be a source of antibiotic-resistant pathogenic bacterial strains despite its enormous medicinal properties, and which is a threat to public health. This work has provided informative data related to the multidrug resistant profile of bacteria of

clinical importance associated with *I. gabonensis* seeds, which could be initiated by the transfer of resistant traits among commensal flora through food chain.

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ORIGINAL RESEARCH ARTICLE

Bio stimulation of indigenous microorganisms with Gomeya: a bioremediation technique

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ABSTRACT

The burden of heavy metals in the environment can be reduced using organic amendment stimulated bacterial remediation. This study employed cattle manure slurry stimulated bacterial inoculum to treat heavy metals-contaminated soil. Samples of contaminated soil and cattle manure were collected from the area surrounding a steel rolling mill and a commercial animal pen respectively. Bacteria were isolated using pour plate technique; identified using various biochemical tests and screened for resistance to heavy metal salts by incorporating heavy metal salts into agar plates. The contaminated soil and manure slurry were analysed for heavy metals and then sterilised separately. Five kilogram of the sterilised contaminated soil was weighed and mixed with 100g of sterilized cow dung slurry and aseptically packed into plastic nursery bags. Bacterial samples showing high tolerance to heavy metal salts were introduced into the bags singly and as a consortium for bioremediation exercise. Thirty-six bacterial isolates were obtained from the contaminated soil. Chemical analysis revealed that the soil was heavily contaminated especially with lead and chromium with concentrations of 1505.1-2333.6 and 1526.0-1678.7 mg/kg, respectively. *Alcaligenes faecalis, Pseudomonas azotoformans* and *Bacillus mycoides* exhibited high tolerance to heavy metals salt and were selected for bioremediation. Post bioremediation analysis of the soil samples revealed a reduction in the concentration of heavy metals concentration with major reduction in the concentration of chromium in groups treated with *P. azotoformans*. Biostimulation of microorganisms with organic amendment effectively remediated heavy metals contaminated soil and can be employed in the treatment of such contaminated environments.

Biological: Microbiology. Keywords: *Cattle manure slurry, biomagnification, stimulated bacterial inoculum*

INTRODUCTION

Some important heavy metals such as cadmium, copper, lead, chromium and mercury are also important environmental contaminants and they are found in high concentration especially in areas with high anthropogenic activities (Suruchi and Khanna, 2011). Though some of these

heavy metals are also soil micronutrients; the extent of soil pollution by these heavy metals and base metal ions is alarming. As a result of pollution, it has been observed that the larger the urban area, the lower the quality of the environment (Eddy, 2004a). The various sources through which heavy metals are released into the soil environment include but are not limited to natural means such as emissions from volcanoes, transport of continental dust and the weathering of metal-enriched rocks (Ernst, 1998) or as a result of various anthropogenic activities such as exploration of mines and smelters, the application of manures, fertilizers, metal based pesticides and metal-enriched sewage sludge in agriculture, combustion of fossil fuels, metallurgical industries, military training, manufacturing, usage and disposal of electronics.

Heavy metal uptake by plants grown on polluted soils has been studied to a considerable extent (Suruchi and Khanna, 2011; Navarro et al., 2008; Dixit et al., 2015; Sukreeyapongse et al., 2002, Yusuf et al., 2003). Heavy metal uptake via roots from contaminated soils and surface water, and direct deposition of heavy metal contaminants from the atmosphere unto plant surfaces can lead to contamination of plant by heavy metals. When these metals exceed the physiological demand of plant, they may not only be toxic to the plants, but oftentimes enter into the food chain, become biomagnified and pose serious health concern to humans (Sugiyama, 1994; Odoh and Kolawole, 2011). The biotoxic effects of heavy metals on plants depend upon the concentrations and oxidation states of heavy metals, its source and mode of deposition (Duruibe et al., 2007).

Though various conventional technologies such as chemical oxidation, precipitation, ion exchange, soil washing, incineration, solidification and stabilization are usually employed in the remediation of heavy metals contaminated soil (FRTR, 2000; Gomes et al., 2012) biological treatment of heavy metal-contaminated soil is often more attractive than direct chemical or physical treatment. One of the most promising technological approaches to the problem of heavy metal contamination in the environment is bioremediation. This can be attributed to the ability of the microorganisms to directly sequester contaminants rather than merely transferring them from one medium to another (USEPA, 1995). Some organisms which have been involved in bioremediation processes include *Pseudomonas aeruginosa, P. ambigua, P. fluorescens,* other species such as *Bacillus cereus, B. subtilis, E. coli* (ATCC 33456), *Achromobacter Eurydice, Micrococcus roseus, Enterobacter cloacae, Desulfovibriode sulfuricans* and *D. vulgaris. Shewanella algae* BrY-MT have been reported to be effective in bioremediation of various contaminants (Guha et al., 2012; Camargo et al., 2003). Comparing the effectiveness of conventional methods of metal removal with biological methods of metal removal, it has been observed that the use of biomass of microorganisms has distinct advantages over conventional methods due to the fact that they are highly selective and cost effective, have diversity of active binding site (Ahluwalia and Goyal, 2007; Green-Ruiz et al., 2008). For instance, microorganisms can assimilate heavy metals actively (bioaccumulation) and/or passively (adsorption) (Hussein et al., 2001). The bacterial cell walls, which consist mainly of polysaccharides, lipids and proteins, offer many functional groups that can bind heavy metal ions, and these include carboxylate, hydroxyl, amino and phosphate groups (Randhawa and Kullar, 2011). Algae, fungi, yeast, protozoa and bacteria have been employed in the removal of heavy metals from industrial waste waters by using the microorganisms in whole and/or using products of their metabolism such as enzymes and biosurfactant (Congeevaram et al., 2007; Özdemir and Kılınc, 2012; Özdemir et al., 2012).

Cattle manure slurry/gomeya usually referred to as a waste product can enhance the degradation of contaminants in the environment. Cattle dung slurry is a cheap and easily available rich source

of organic amendment. It is a mixture of cattle dung and urine in a ratio of around 3:1 respectively (Randhawa and Kullar, 2011). According to Adedokun and Ataga(2007), soil amendments or additives are needed to increase the activities of microbes and for effective bioremediation of polluted soil.

Corchorus olitorius commonly called jute or Jew mallow belongs to the Tiliaceae family. The choice of *C. olitorius* for this study is based on the fact that it is one of the most popular vegetables in every home; hence it is grown in nearly all home gardens, market gardens near the city and truck gardens around the world (Aluko et al., 2014).

The aim of this study was to bioremediate heavy metals contaminated soil using organic amendment stimulated bacterial remediation. This study was therefore designed to study the ability of bacteria isolated from heavy metal-contaminated soil to effectively remove heavy metals in order that the bioremediated soil can support plant growth.

Materials and Methods

Sample collection

The study location was densely contaminated with heavy metals contained in effluent released as a result of the activities of a steel rolling company in South-western Nigeria. Consequently, plants grown in the vicinity of the company could not thrive which had an impact on the livelihood of people living in that area as majority of them were peasant farmers. The study location was visited during the dry and rainy season to carry out *in situ* analysis in order to obtain background knowledge of the seasonal variations in the physical and chemical properties of the location. Physical observation was done to observe changes in physical characteristics such as colour, texture, odour and deposition of effluents from the rolled steel industry which were considered as indicators for pollution. Soil sample was collected from different points of the location using a soil auger. The soil was transported to the Department of Microbiology, University of Ibadan where microbiological and chemical analysis were conducted within 24 h of collection.

Analysis of heavy metal-contaminated soil

The soil samples collected were thoroughly mixed using a hand trowel to obtain a composite sample before subjecting it to various analyses. Soil hydrogen ion concentration (pH 1:1 H_2O) was determined using a glass electrode pH meter (Hanna instruments HI2210) following the methods described by Bates (1954). The exchangeable acidity was determined using the KCl extraction method following the method of Mclean (1965), the organic matter in the soil sample in the form of carbon was determined using the Walkey-Black wet oxidation method as described by Page (1982), the total nitrogen in the composite soil sample was determined using the macro-Kjeldahl method as described by Page (1982), the phosphorus in the composite soil sample was analysed using the vanado-molybdate method (AOAC, 2012). The calcium and magnesium content of the soil was determined using methods described by Mehlich (1953) and Watanabe and Olsen (1965). The concentration of heavy metals such as cadmium, iron, copper, lead, chromium, zinc, nickel, manganese and cobalt present in the soil sample was determined using the wet digestion procedure (SSSA, 1971).This was carried out by weighing 0.5 g of the 0.5 mm sieved soil into a 100 mL Berzellius beaker, 5 mL HNO_3 and 2 mL $HClO_4$ was added and covered with a watch glass. This was digested in a fume cupboard by heating it to a final volume of 3 to 5 mL. Ten to fifteen millilitres of water was added and the digest solution was

filtered through an acid washed filter paper into a 50 mL volumetric flask. It was diluted to volume with deionized water and the filter paper was washed with water. The filtrate was used to determine the concentration of heavy metals present in the sample using Buck Scientific 210/211 VGP Atomic Absorption Spectrophotometer (AAS).

Isolation of microorganisms

The determination of the total viable bacteria count (TVBC) was carried out in triplicates. The agar medium and the diluents used were sterilized at 121^{o}C for 15 minutes. One gram of the thoroughly mixed composite soil samples was suspended in 9 mL of sterile distilled water and serially diluted (Olutiola et al., 2000). One millilitre of appropriate dilutions was inoculated into sterile Petri dishes and already prepared and cooled nutrient agar (Lab M, United Kingdom) was added to it using the pour plate technique as described by Olutiola et al. (2000). Inoculated plates were incubated at 37^{o}C for 24 h after which distinct bacteria colonies were counted. Morphologically distinct bacteria colonies were subcultured by streaking on fresh nutrient agar plates until pure bacteria colonies were obtained. Pure cultures of each bacteria strain were stored on nutrient agar slants at 4 ^{o}C for further studies. Pure bacterial isolates were subjected to various biochemical tests to aid their identification.

Molecular Characterisation of Bacterial Isolates

16S rRNA based identification

Isolation of 16S rRNA gene of the bacterial isolates were carried out using QIAamp DNA Mini Kit (250) cat no 51306 after which the sequences were amplified using Applied Biosystems Thermocycler, model 9800. Sequencing of the 16S rRNA was carried out using a 16-well Applied Biosystems sequencing plate following the manufacturer's instructions. The obtained sequences of bacterial 16S rRNA were analysed using Sequence Scanner (Applied Biosystems) software and the 16S rRNA sequence contigs were generated using Chromas Pro. The online program BLASTn was used to find out the related sequences with known taxonomic information in the databank at NCBI website (http://www.ncbi.nlm.nih.gov/BLAST) to accurately identify the bacterial strain. The data obtained from the molecular characterization was used in constructing a phylogenic tree for the bacterial isolates and also submitted to DNA Data Bank of Japan (DDBJ) for accession numbers.

Phylogenetic Analyses of bacterial strains

The 16S rRNA gene sequences obtained from the GenBank database of the National Centre for Biotechnology Information (NCBI) were aligned using the Molecular Evolutionary Genetics Analysis (MEGA) software version 6 following the method described by Hall (2013) and Tamura et al. (2013). The evolutionary history of the bacterial isolates was inferred using the Neighbor-Joining method as described by Saitou and Nei (1987). The tree was drawn to scale, with branch lengths in the same units as those of the evolutionary distances used to infer the phylogenetic tree. The evolutionary distances were computed using the Maximum Composite Likelihood method (Tamura et al., 2004) and in the units of the number of base substitutions per site.

Determination of the susceptibility of bacterial isolates to heavy metal salts

The susceptibility of the bacterial isolates to increasing concentration of chromium, cadmium, lead, copper, cobalt, nickel and zinc was determined quantitatively using the agar diffusion method following the method described by Narasimhulu *et al.* (2010). Concentration of heavy metals in nutrient agar medium was gradually increased from 100-500 µg/mL. The screening was done by streaking a 24 h old culture of the test organism on nutrient agar plate supplemented with 100 µg/mL of the salt of the heavy metals of interest and was incubated for five days. Isolates that grew at this concentration were subcultured to nutrient agar plates supplemented with higher concentration of the heavy metal salts until 500 µg/mL concentration of heavy metal at increasing level of 50µg/mL was attained. Isolates which were observed to have high tolerance to heavy metal salts were maintained on agar slants and stored at 4 ^{o}C to be used for bioremediation of the contaminated soil samples.

Sterilization of soil sample

Composite soil samples collected from the heavy metal-contaminated steel rolling site were air dried in the sunlight for a day and then sieved using a 0.5 mm nylon mesh sieve. The soil sample was then sequentially sterilized using hot air oven at 105 ^{o}C for one hour, after which it was aseptically packaged by weighing 5 kg into sterile polythene bags for the planting exercise as described by Saeed and Rafique(1980) and Iqbal *et al.*(2011). To check for sterility of the soil sample, the soil sample was subjected to the method used for isolation of microorganisms as described above.

Collection of Corchorus olitorius seeds

For the purpose of this study, seeds of *Corchorus olitorius* already treated with scarification method (in order to break the dormancy) were obtained from Agronomy Department, Faculty of Agriculture, University of Ibadan, Nigeria.

Bioremediation of contaminated soil sample

Five kilogram of already sterilized soil sample were aseptically weighed and mixed with one hundred gram of sterilized cow manure slurry. This was then packed into perforated polythene bags (nursery bags) to be used for the bioremediation and planting exercise. The bioremediation and planting exercises were conducted in a screen house. Working solution for the bioremediation exercise was prepared using a modified method of Ayotamuno *et al.* (2009) by inoculating each of the bacterial isolates into peptone water broth and incubating until a cell density of 7.6×10^{11}cfu/mL was obtained, however, for bioremediation exercise using mixed culture, the working solution was allowed to reach a cell density of 1.5×10^{12}cfu/ml as described by Okparanma *et al*. (2009). Twenty millilitres of the working solution of the bacterial isolate was pipetted into each experimental setup. The experiment was laid out in a Completely Randomized Design (CRD) having eight treatments with five replicates making a total of forty pots.

Treatments:

A Sterilized soil treated with *Alcaligenes faecalis* (LC349889.1) and manure

B Sterilized soil treated with *Pseudomonas azotoformans* (LC349894.1) and manure

C Sterilized soil treated with *Bacillus mycoides* (LC349697.1) and manure

AB Sterilized soil treated with consortia of *Alcaligenes faecalis* (LC349889.1) and *Pseudomonas azotoformans* (LC349894.1) and manure

AC Sterilized soil treated with consortia of *Alcaligenes faecalis* (LC349889.1) and *Bacillus mycoides* (LC349897.1) and manure

BC Sterilized soil treated with consortia of *Pseudomonas azotoformans* (LC349894.1) and *Bacillus mycoides* (LC349897.1) and manure

ABC Sterilized soil treated with consortia of *Alcaligenes faecalis* (LC349889.1), *Pseudomonas azotoformans* (LC349894.1) and *Bacillus mycoides* (LC349897.1) and manure

Control Sterilized soil alone (without bacterial inoculums and manure).

Planting of *Corchorus olitorius*

Planting of *C. Olitorius* was done immediately after introducing the bacterial isolates. The pots were watered twice daily (100 mL/time) for the first two weeks of planting and later reduced to once daily so as to prevent the leaching of nutrient from the treatments. This was done till the experiment was terminated seven weeks after planting (WAP). Plant height was measured from the rhizoplane to the apical tip of the plant in centimetres starting from 2WAP till the seventh week when the experiment was terminated, stem diameter measured using a vernier caliper below the first nodes of the plant and number of leaves obtained by counting the number of leaves per plant manually Elings (2000).

Analysis of harvested C. olitorius

At the end of the experiment, the plants were harvested by uprooting the plantings and pooled together per treatment group. The roots were washed to remove attached soil debris and then subjected to various analyses. The wet and dry weight, moisture content, dry matter, % crude protein, % ash, % ether extract (fat), % crude fibre were determined using standard analytical methods as described by AOAC (2012). The heavy metal content of the plant samples was determined following the method described by SSSA (1971) using Buck Scientific 210/211 VGP Atomic Absorption Spectrophotometer (AAS).

Analysis of the bioremediated soil samples

Upon termination of the experiment and harvest of the plants, the five soil replicates in each treatment group were pooled together and mixed well in order to obtain a composite sample which was analysed for pH, nitrogen, organic carbon, exchangeable acidity, available phosphorus, calcium, potassium, sodium, magnesium, manganese, iron, copper, zinc, lead, cadmium, chromium, cobalt and nickel content following the methods described earlier.

Data Analysis

Data obtained were analysed and reported as mean ± standard deviation of five measurements and analysed using univariate analysis of variance and Duncan Post Hoc test to determine significant differences ($p \geq 0.05$) between treatments using Statistical Package for Social Science Research version 17 (SPSS).

Results

The collected soil samples were dark brown in colour with a characteristic choking odour which is peculiar to heavy metal contaminated sites. Table 1 shows the result of the initial *in situ* analysis carried out to determine the physical, chemical and heavy metals properties of the composite soil samples collected during the rainy and dry season. It was observed that the soil from the study site had higher physical, chemical and heavy metal content during the dry season than the rainy season. For instance, heavy metals such as cadmium, lead, cobalt, nickel and chromium had concentrations of 3.0, 2333.6, 13.7, 40.6 and 1678.7 mg/kg, respectively during the dry season and concentrations of 0.5, 1505.5, 10.5, 31.5 and 1526.0 mg/kg, respectively during the rainy season.

Table 1: Physical and chemical properties of the heavy metals contaminated soil sample during dry and rainy seasons

Parameters	Dry season	Rainy season
Ph	6.5	7.0
Exchangeable Acidity(meq/100g)	0.4	0.4
Mineral content		
Total organic carbon (TOC) (g/kg)	42.4	40.8
Total Nitrogen (g/kg)	4.0	3.9
Available Phosphorus(mg/kg)	14.0	13.3
Ca (cmol/kg)	2.6	2.7
K (cmol/kg)	0.3	0.4
Na (cmol/kg)	0.5	0.3
Mg (cmol/kg)	0.5	0.4
Heavy metals (mg/kg)		
Mn	99.4	98.5
Fe	24.8	23.8
Cu	2.8	1.0
Zn	2.6	1.4
Cd	3.0	0.5
Pb	2333.6	1505.5
Co	13.7	10.5
Ni	40.6	31.5
Cr	1678.7	1526.0

Thirty six bacterial isolates were obtained from the contaminated soil sample and they were distributed as follows: *Pseudomonas* sp (52.77%), Proteus *mirabilis* (13.89%), *Alcaligenes faecalis* (13.89%), *Enterobacter* sp (8.33%), *Providencia* sp (5.56%) and *Bacillus* sp (5.56%).

However only three of these isolatesviz: *Alcaligenes faecalis* (LC349889.1), *Pseudomonas azotoformans* (LC349894.1) and *Bacillus mycoides* (LC349897.1) exhibited high tolerance to heavy metal salts, therefore the three bacterial isolates were selected for bioremediation exercise. Figures 1-3 shows the phylogenetic tree constructed for each of the isolates based on the molecular data obtained when they were identified using molecular characterization (isolates appear in bold format).

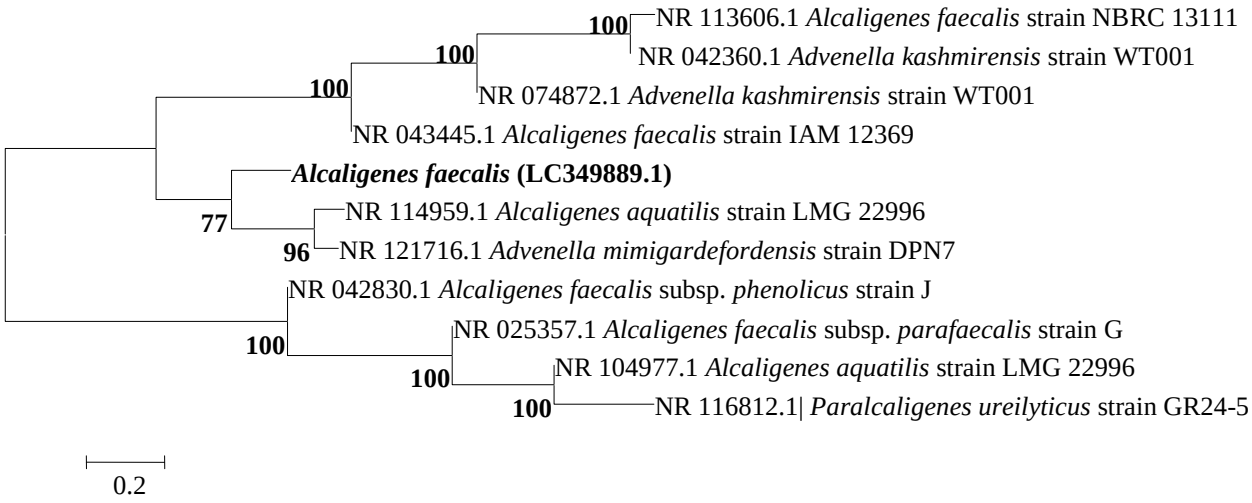

Figure 1: Evolutionary relationship of *Alcaligenes faecalis* (LC349889.1)

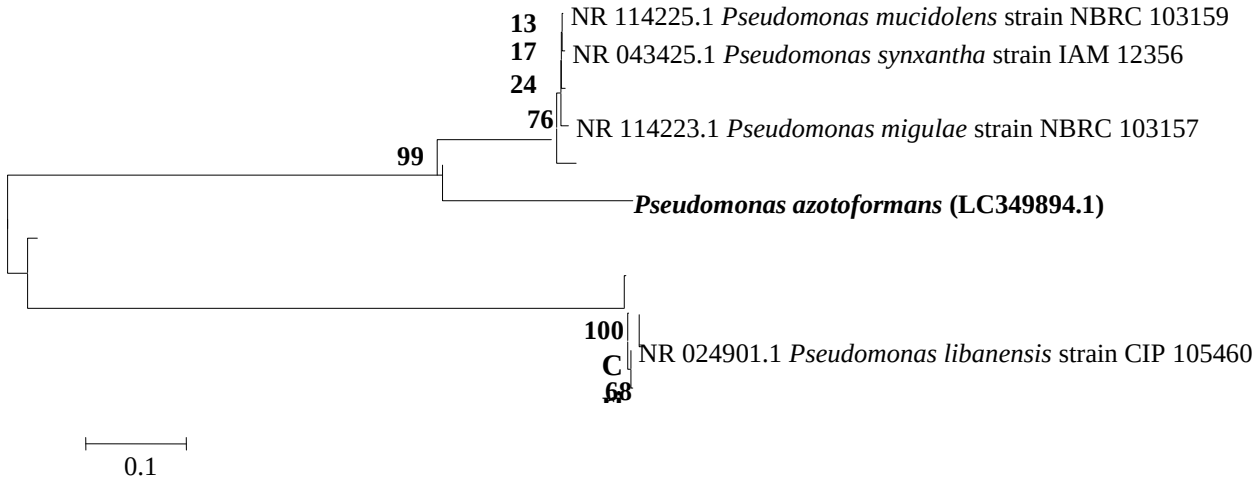

Figure 2: Evolutionary relationship of *Pseudomonas azotoformans*(LC349894.1)

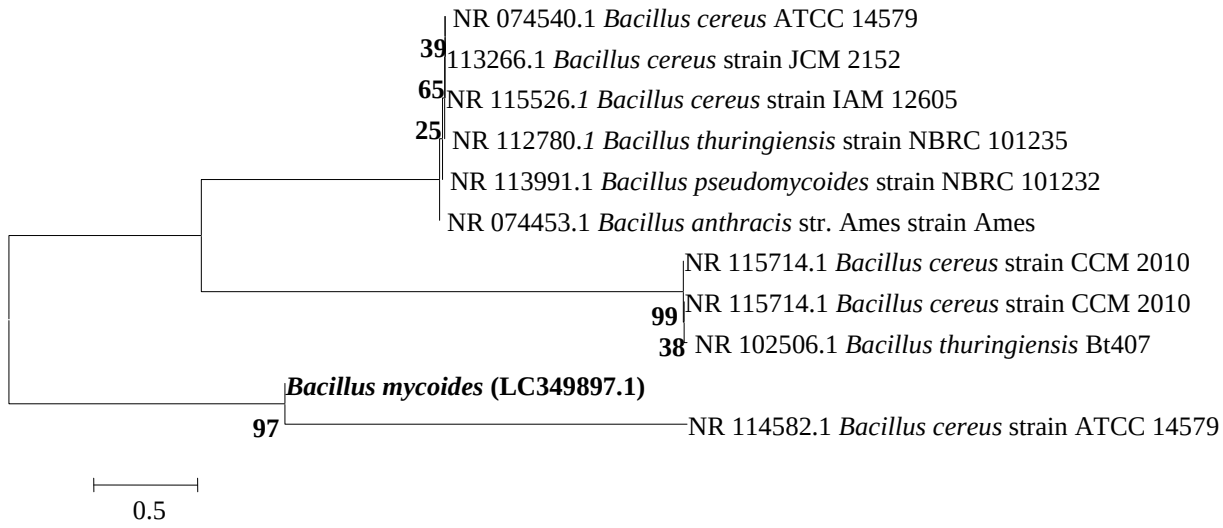

Figure 3: Evolutionary relationship of *Bacillus mycoides* (LC349897.1)

The result obtained for the monitored agronomic parameters is as shown in Figures 4-6. There was significant difference (at p≥0.05) in the plant height of the treatments starting from 2WAP to 7WAP when the experiment was terminated as shown by Figure 4. It was observed that the plant response in the various treatment groups varied in their performance. It was observed that *C. Olitorius* harvested from soil bioremediated with a mixed culture of *Pseudomonas azotoformans* (LC349894.1)and *Bacillus mycoides* (LC349897.1) had the least height of 2.76±0.15 cm at 2 WAP, however at 6 WAP, the highest height was observed in *C. Olitorius* harvested from soil bioremediated with a consortia of *Alcaligenes faecalis* (LC349889.1), *Pseudomonas azotoformans* (LC349894.1) and *Bacillus mycoides* (LC349897.1) with a height of 7.84±0.69 cm, while the least height was observed in *C.olitorius* harvested from soil bioremediated with *Pseudomonas azotoformans* (LC349894.1) having a height of 4.68±0.41 cm, and this trend was maintained till the experiment was terminated. The control had the highest height of 4.10±0.55 cm as at 2WAP and at 7WAP it had a height of 5.86±0.57 cm.

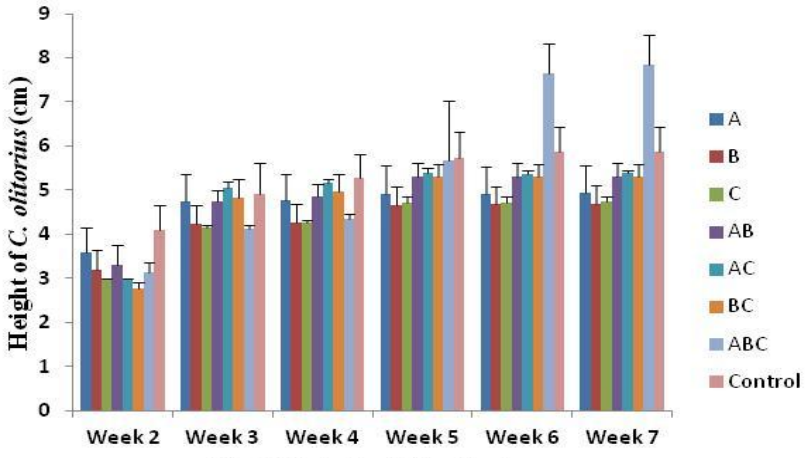

Fig 4: Height of *C. olitorius*

Keys: A- *C. Olitorius* harvested from sterilized soil bioremediated with *Alcaligenes faecalis* (LC349889.1), B- *C. Olitorius* harvested from sterilized soil bioremediated with *Pseudomonas azotoformans*(LC349894.1), C- *C. Olitorius* harvested from sterilized soil bioremediated with *Bacillus mycoides* (LC349897.1), AB- *C. Olitorius* harvested from sterilized soil bioremediated with mixed culture of *Alcaligenes faecalis* (LC349889.1) and *P. Azotoformans* (LC349894.1), AC- *C. Olitorius* harvested from sterilized soil bioremediated with mixed culture of *Alcaligenes faecalis* (LC349889.1) and *Bacillus mycoides* (LC349897.1), BC- *C. Olitorius* harvested from sterilized soil bioremediated with mixed culture of *P. Azotoformans* (LC349894.1) and *Bacillus mycoides* (LC349897.1), ABC- *C. Olitorius* harvested from sterilized soil bioremediated with consortia of *Alcaligenes faecalis* (LC349889.1), *Pseudomonas azotoformans* (LC349894.1) and *Bacillus mycoides* (LC349897.1), Control- *C. Olitorius* harvested from sterilized soil alone.

The results obtained for number of leaves/plant revealed that there was significant difference (at $p \leq 0.05$) in the number of leaves per plant among the treatments starting from 2WAP as shown by Figure 5. *C. Olitorius* harvested from soil bioremediated with *Alcaligenes faecalis* (LC349889.1) had the highest number of leaves/plant from 2WAP till 4WAP having an average of 4.00±0.00 and 5.20±0.45, respectively but by 5WAP till the 7WAP when the experiment was terminated, *C. Olitorius* harvested from soil bioremediated with a consortia of *Alcaligenes faecalis* (LC349889.1), *Pseudomonas azotoformans* (LC349894.1) and *Bacillus mycoides* (LC349897.1) had the highest number of leaves/plant with an average of 5.80±0.45.

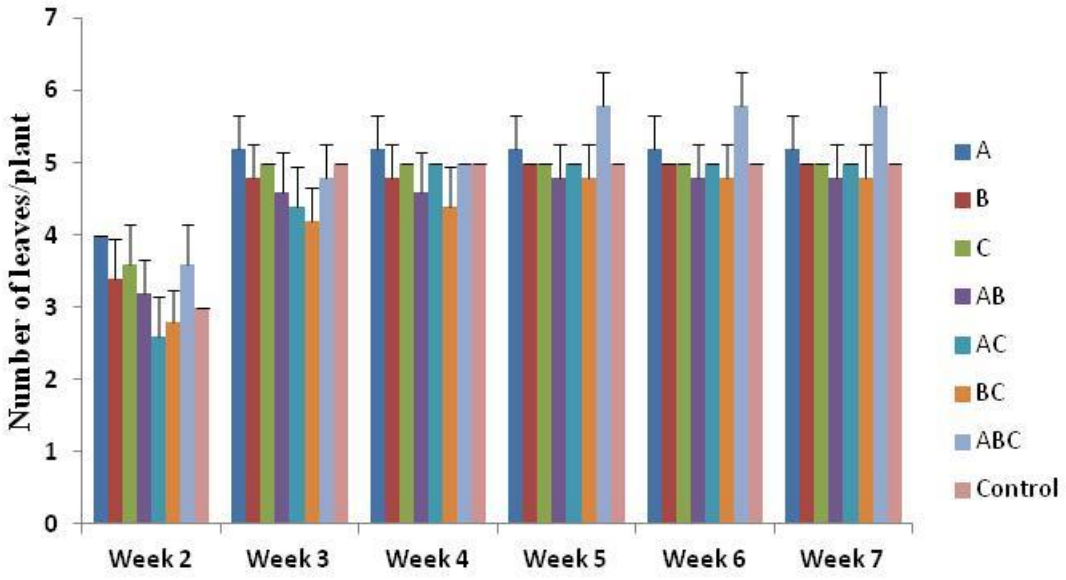

Fig 5: Number of leaves/*C. olitorius*

Keys: A- *C. olitorius* harvested from sterilized soil bioremediated with *Alcaligenes faecalis* (LC349889.1), B- *C. olitorius* harvested from sterilized soil bioremediated with *Pseudomonas azotoformans* (LC349894.1), C- *C. olitorius* harvested from sterilized soil bioremediated with *Bacillus mycoides* (LC349897.1), AB- *C. olitorius* harvested from sterilized soil bioremediated with mixed culture of *Alcaligenes faecalis* (LC349889.1) and *P. azotoformans* (LC349894.1), AC- *C. olitorius* harvested from sterilized soil bioremediated with mixed culture of *Alcaligenes faecalis* (LC349889.1) and *Bacillus mycoides* (LC349897.1), BC- *C. olitorius* harvested from sterilized soil bioremediated with mixed culture of *P.azotoformans* (LC349894.1) and *Bacillus*

mycoides (LC349897.1), ABC- *C. olitorius* harvested from sterilized soil bioremediated with consortia of *Alcaligenes faecalis* (LC349889.1), *Pseudomonas azotoformans* (LC349894.1) and *Bacillus mycoides* (LC349897.1), Control- *C. olitorius* harvested from sterilized soil alone.

There was significant difference at $p \leq 0.05$ in the plant stem diameter of harvested *C. Olitorius* starting from 2WAP till 7WAP except at 3WAP as shown in Figure 6. By 7WAP, the highest stem diameter was observed in *C. Olitorius* harvested from soil bioremediated with a consortia of *Alcaligenes faecalis* (LC349889.1), *Pseudomonas azotoformans* (LC349894.1) and *Bacillus mycoides* (LC349897.1) which had an average of 1.35±0.00 mm while the smallest stem diameter was observed in *C. Olitorius* harvested from soil bioremediated with *Pseudomonas azotoformans* (LC349894.1), mixed culture of *Alcaligenes faecalis* (LC349889.1) and *Pseudomonas azotoformans* (LC349894.1), mixed culture of *Alcaligenes faecalis* (LC349889.1) and *Bacillus mycoides* (LC349897.1) and mixed culture of *Pseudomonas azotoformans* (LC349894.1) and *Bacillus mycoides* (LC349897.1) with each having an average of 1.20±0.00 mm each.

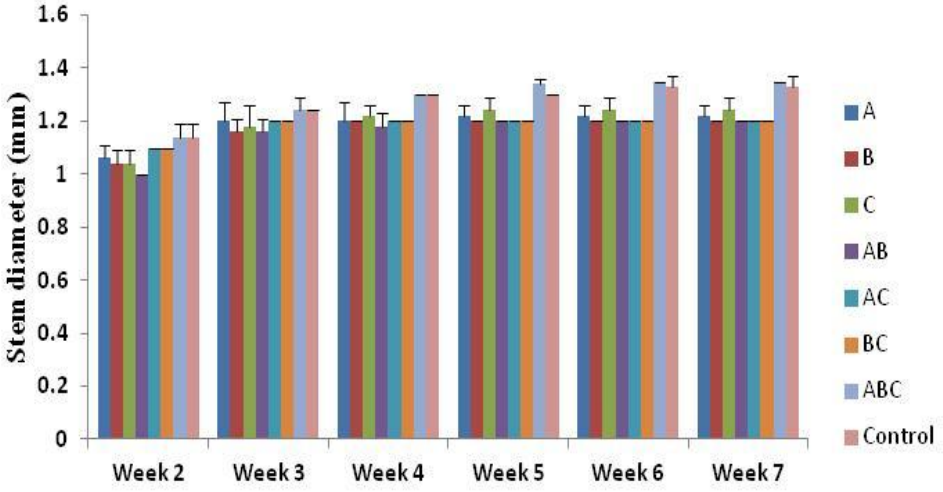

Fig 6: Stem diameter/ *C. olitorius*

Keys: A- *C. olitorius* harvested from sterilized soil bioremediated with *Alcaligenes faecalis* (LC349889.1), B- *C. olitorius* harvested from sterilized soil bioremediated with *Pseudomonas azotoformans* (LC349894.1), C- *C. olitorius* harvested from sterilized soil bioremediated with *Bacillus mycoides* (LC349897.1), AB- *C. olitorius* harvested from sterilized soil bioremediated with mixed culture of *Alcaligenes faecalis* (LC349889.1) and *P. azotoformans* (LC349894.1), AC- *C. olitorius* harvested from sterilized soil bioremediated with mixed culture of *Alcaligenes faecalis* (LC349889.1) and *Bacillus mycoides* (LC349897.1), BC- *C. olitorius* harvested from sterilized soil bioremediated with mixed culture of *P.azotoformans* (LC349894.1) and *Bacillus mycoides* (LC349897.1), ABC- *C. olitorius* harvested from sterilized soil bioremediated with consortia of *Alcaligenes faecalis* (LC349889.1), *Pseudomonas azotoformans* (LC349894.1) and *Bacillus mycoides* (LC349897.1), Control- *C. olitorius* harvested from sterilized soil alone.

Tables 2 and 3 shows the result obtained for the post-bioremediation and harvest analysis harvested of proximate matter and heavy metals content of *C. olitorius* respectively. It was observed that there were significant differences in the responses of the various treatment groups.

Table 2: Proximate matter of harvested *C. olitorius*

Treatment	Proximate matter							
	Fresh weight (g)	Dry weight (g)	% Moisture	Dry matter	% Crude protein	% Ash	% Ether extract (fat)	% Crude fibre
A	0.22±0.05^{c}	0.10±0.01^{f}	55.00±7.07^{g}	45.00±7.07^{h}	12.24±1.90^{i,j}	19.06±1.47^{o}	1.40±0.15^{s}	22.62±0.55^{t}
B	0.26±0.03^{c}	0.10±0.03^{f}	61.90±6.79^{g}	38.10±6.79^{h}	7.92±1.17^{jkl}	20.08±0.11^{no}	1.66±00.22^{rs}	19.78±0.53^{vw}
C	0.36±0.09^{c}	0.14±0.04^{f}	62.00±0.14^{g}	38.00±0.14^{h}	15.82±1.90^{i}	14.97±0.69^{p}	1.93±0.25^{qr}	18.72±0.25^{w}
AB	0.33±0.13^{c}	0.15±0.08^{f}	56.25±8.84^{g}	43.75±8.84^{h}	10.39±3.18^{j,k}	17.88±1.62^{o}	1.91±0.15^{qr}	19.62±0.54^{vw}
AC	0.38±0.05^{c}	0.14±0.01^{f}	61.10±3.25^{g}	38.90±3.25^{h}	11.03±2.08^{j}	18.16±1.48^{o}	1.35±0.22^{s}	18.95±0.96^{vw}
BC	0.29±0.07^{c}	0.13±0.04^{f}	51.95±26.36^{g}	48.05±26.38^{h}	6.58±0.03^{k,l}	21.65±0.78^{n}	2.00±0.05^{qr}	19.88±0.47^{vw}
ABC	2.54±0.57^{a}	0.99±0.17^{d}	60.75±2.05^{g}	39.25±2.05^{h}	4.44±1.33^{l}	24.57±0.62^{m}	2.00±0.20^{q,r}	21.72±0.99^{tu}
Control	1.10±0.13^{b}	0.63±0.24^{e}	43.65±15.34^{g}	56.35±15.34^{h}	5.57±0.80^{l}	22.56±0.63^{mn}	2.11±0.06^{q}	20.61±0.78^{uv}

******values with the same letters on each column are not significantly different from each other at p≤0.05***

Keys: A- *C. olitorius* harvested from sterilized soil bioremediated with *Alcaligenes faecalis* (LC349889.1), B-*C. olitorius* harvested from sterilized soil bioremediated with *Pseudomonas azotoformans* (LC349894.1), C- *C. olitorius* harvested from sterilized soil bioremediated with *Bacillus mycoides* (LC349897.1), AB- *C. olitorius* harvested from sterilized soil bioremediated with mixed culture of *Alcaligenes faecalis* (LC349889.1) and *P. azotoformans* (LC349894.1), AC- *C. olitorius* harvested from sterilized soil bioremediated with mixed culture of *Alcaligenes faecalis* (LC349889.1) and *Bacillus mycoides* (LC349897.1), BC- *C. olitorius* harvested from sterilized soil bioremediated with mixed culture of *P.azotoformans* (LC349894.1) and *Bacillus mycoides* (LC349897.1), ABC- *C. olitorius* harvested from sterilized soil bioremediated with consortia of *Alcaligenes faecalis* (LC349889.1), *Pseudomonas azotoformans* (LC349894.1) and *Bacillus mycoides* (LC349897.1), Control- *C. olitorius* harvested from sterilized soil alone.

Table 3: Heavy metal content of harvested *C. olitorius*

Treatment	Heavy metals							
	Iron	Copper	Zinc	Cadmium	Lead	Cobalt	Chromium	Nickel
A	25.35±0.49 ^{c}	36.05±2.05^{d}	17.45±1.34^{g}	35.55±8.56^{i}	37.70±0.85^{l}	30.50±1.56^{n}	27.00±0.71^{p}	29.00±13.29^{s}
B	26.95±0.64^{bc}	31.70±2.26^{de}	16.50±0.85^{g}	36.45±4.60^{i}	37.65±5.02^{l}	27.70±1.41^{n}	20.75±1.48^{q}	29.00±14.57^{s}
C	28.40±1.70^{b}	29.15±1.34^{de}	17.00±0.28^{g}	32.95±6.43^{i}	34.55±2.47^{l}	28..35±6.86^{n}	24.80±3.82^{pq}	26.70±8.63^{s,t}
AB	24.95±1.77^{c}	30.05±3.60^{de}	16.10±0.85^{g}	34.85±8.13^{i}	36.30±4.81^{l}	29.35±1.06^{n}	24.40±2.97^{pq}	29.35±11.52^{s}
AC	28.35±1.77^{b}	30.55±6.01^{de}	15.90±0.71^{g}	27.85±11.67^{i,j}	32.50±2.55^{l}	28.55±1.48^{n}	26.40±2.40^{p}	27.70±9.76^{s,t}
BC	28.45±049^{b}	31.00±3.68^{de}	16.10±0.85^{g}	12.00±12.02^{jk}	31.85±1.77^{l}	26.80±0.99^{n}	26.50±2.55^{p}	31.25±11.10^{s}
ABC	37.95±1.20 ^{a}	20.20±4.95^{f}	4.25±0.21^{h}	4.40±0.28^{k}	10.90±0.85^{m}	6.30±0.71^{o}	4.15±0.64^{r}	3.20±0.85^{t}
Control	38.10±0.85^{a}	25.80±0.85^{ef}	5.95±0.78^{h}	4.80±0.71^{k}	9.15±0.64^{m}	5.90±0.71^{o}	4.00±0.14^{r}	3.25±0.63^{t}

******values with the same letters on each column are not significantly different from each other at p≤0.05***

Keys: A- *C. olitorius* harvested from sterilized soil bioremediated with *Alcaligenes faecalis* (LC349889.1), B- *C. olitorius* harvested from sterilized soil bioremediated with *Pseudomonas azotoformans* (LC349894.1), C- *C. olitorius* harvested from sterilized soil bioremediated with *Bacillus mycoides* (LC349897.1), AB- *C. olitorius* harvested from sterilized soil bioremediated with mixed culture of *Alcaligenes faecalis* (LC349889.1) and *P. azotoformans* (LC349894.1), AC- *C. olitorius* harvested from sterilized soil bioremediated with mixed culture of *Alcaligenes faecalis* (LC349889.1) and *Bacillus mycoides* (LC349897.1), BC- *C. olitorius* harvested from sterilized soil bioremediated with mixed culture of *P.azotoformans* (LC349894.1) and *Bacillus mycoides* (LC349897.1), ABC- *C. olitorius* harvested from sterilized soil bioremediated with consortia of *Alcaligenes faecalis* (LC349889.1), *Pseudomonas azotoformans* (LC349894.1) and *Bacillus mycoides* (LC349897.1), Control- *C. olitorius* harvested from sterilized soil alone.

Table 4 shows the result obtained for heavy metal content of the soil after bioremediation and harvesting of *C. Olitorius* have been conducted. It was observed that the concentration of heavy metals present in the soil was significantly reduced compared to the concentrations observed in the contaminated soil during rainy and dry seasons.

Table 4: Physical and chemical properties of bioremediated soil after harvesting *C. olitorius*

Parameters	A	B	C	AB	AC	BC	ABC	CONTROL
pH	8.50	8.41	8.52	8.49	8.50	8.41	8.51	8.44
Exchangeable Acidity (meq/100g)	0.40	0.30	0.50	0.40	0.50	0.30	0.40	0.40
Mineral content								
T.O.C (g/kg)	63.52	59.98	65.11	60.74	61.93	67.89	67.09	63.92
T/N (g/kg)	6.57	6.20	6.74	6.28	6.41	7.02	6.94	6.61
Available Phosphorus (mg/kg)	42.87	33.20	66.48	39.30	39.78	31.46	25.70	49.67
Ca (Cmol/kg)	86.20	105.66	128.11	107.41	99.80	125.62	94.84	101.42
K (Cmol/kg)	2.75	2.78	14.32	2.06	2.42	1.99	0.97	6.04
Na (Cmol/kg)	8.04	8.70	12.39	10.22	9.13	8.91	9.35	8.91
Mg (Cmol/kg)	0.53	1.17	1.08	1.17	0.86	1.23	0.90	1.19
Heavy metals (mg/kg)								
Mn (mg/kg)	450.0	515.0	738.0	867.0	581.0	756.0	558.0	910.0
Fe (mg/kg)	17.4	13.5	17.2	15.2	10.2	12.1	14.1	23.5
Cu (mg/kg)	4.12	1.61	2.04	0.97	1.07	2.01	1.51	5.84
Zn (mg/kg)	3.84	1.04	1.68	1.12	0.93	1.68	0.99	4.92
Cd (mg/kg)	2.2	2.6	2.6	2.2	1.8	2.1	2.0	4.8
Pb (mg/kg)	21.3	20.8	40.5	24.3	21.1	20.8	22.6	1360.56
Co (mg/kg)	1.04	1.51	1.68	1.86	1.04	1.33	1.75	8.72
Ni (mg/kg)	2.23	1.06	0.93	1.22	0.86	1.04	1.43	1.21
Cr (mg/kg)	1.96	1.81	3.36	3.04	2.17	2.23	3.06	1240.87

Keys: A- *C. olitorius* harvested from sterilized soil bioremediated with *Alcaligenes faecalis* (LC349889.1), B- *C. olitorius* harvested from sterilized soil bioremediated with *Pseudomonas azotoformans* (LC349894.1), C- *C. olitorius* harvested from sterilized soil bioremediated with *Bacillus mycoides* (LC349897.1), AB- *C. olitorius* harvested from sterilized soil bioremediated with mixed culture of *Alcaligenes faecalis* (LC349889.1) and *P. azotoformans* (LC349894.1), AC- *C. olitorius* harvested from sterilized soil bioremediated with mixed culture of *Alcaligenes faecalis* (LC349889.1) and *Bacillus mycoides* (LC349897.1), BC- *C. olitorius* harvested from sterilized soil bioremediated with mixed culture of *P.azotoformans* (LC349894.1) and *Bacillus mycoides* (LC349897.1), ABC- *C. olitorius* harvested from sterilized soil bioremediated with consortia of *Alcaligenes faecalis* (LC349889.1), *Pseudomonas azotoformans* (LC349894.1) and *Bacillus mycoides* (LC349897.1), Control- *C. olitorius* harvested from sterilized soil alone.

Discussion

The area surrounding the study site was heavily contaminated with heavy metals as a result of the release of effluent by a steel rolling industry in the area into the surrounding soil. It was observed that the soil could not support plant growth and some plants which could adapt to the heavy metal in the environment were either drying up or had a yellow colour; all these deviations from normal physical characteristics of a healthy soil and plant indicated a high degree of

pollution of the study area by heavy metals. This is in agreement with the report of Revathi *et al.* (2011), who reported that plant growth is not sustained in soils heavily polluted with heavy metals. The observed deviations in the physical characteristics of the soil samples were in agreement with changes observed in a soil contaminated by acid mine drainage water as reported by Bitala *et al.* (2009).

Analysis of the contaminated soil sample done before treatment revealed that the concentration of the heavy metals were higher during the dry season than during the wet or rainy season. This can be attributed to the effect of leaching which is more evident during the wet or rainy season compared to the dry season and also the rates of deposition of suspended particles are generally higher during the dry season compared to the wet or rainy season. This is in agreement with the findings of Kilicel (1999).

Concentrations of some heavy metals in the contaminated soil were found to exceed the concentrations recommended by WHO and USEPA. For instance, heavy metals such as cadmium, lead, chromium and cobalt had concentrations of 0.50-2.98 mg/kg, 1505.50-2333.55 mg/kg, 1526.00-1678.67 mg/kg and 10.50-13.65 mg/kg, respectively which exceeded the recommended concentrations of 0.003, 10, 2, and 8 mg/kg by WHO and USEPA for each of the respective heavy metals (Parizanganeh *et al*., 2012; Ezejiofor *et al*., 2013); whereas minerals such as calcium, potassium, magnesium and sodium had a concentration of 2.56-2.68, 0.34-0.36, 0.41-0.50 and 0.30-0.50 cmol/kg respectively which were below the recommended values of 10-20, 0.6-1.2, 3-8 and 0.7-1.2 cmol/kg (Parizanganeh *et al*., 2012; Ezejiofor *et al*., 2013). These minerals are essential to plant growth and development, a reduction in their bioavailability often leads to reduced plant growth. A major reason for the reduction in bioavailability of these important minerals could be attributed to the high contamination of the soil with heavy metals. According to Chibuike and Obiora (2014), the presence of heavy metals in a soil may affect the availability of other element especially the minerals in the soil.

The result of the molecular characterization of the isolates used in this study revealed high similarity between the isolated organisms and other related organisms in their genus as revealed by the evolutionary tree. The pH of the treated soil obtained in this study ranged between 8.41 and 8.52, this was suitable for the growth and yield of *C. olitorius*. According to Facciola (1990), *C. Olitorius* is able to grow well in acid, neutral and basic (alkaline) soils; it tolerates soil pH of 4.5 to 8.0. However, extreme pH conditions have the tendency to reduce the availability of iron in the soil which can cause yellowing between leaf veins (Palada and Chang, 2003).

A mean plant height of 7.84 cm was observed in the group treated with the consortia of was *Alkaligenes faecalis* (LC349889.1), *Pseudomonas azotoformans* (LC349894.1) and *Bacillus mycoides* (LC349897.1), at 7 WAP, this height low compared to that obtained by Ogunrinde and Fasimirin (2011) in which they obtained a mean height of 105.03 cm at 7WAP for *C. Olitorius* planted in an uncontaminated soil. The stunted growth of plants observed in this study may be as a result of the effect of heavy metals contamination on the soil as indicated by Kabir *et al.* (2009). Adenipekun *et al.* (2013) reported similar observation in a study in which they observed a height range of 4.083-11.183 cm in *C. Olitorius* grown on a soil contaminated with oil and remediated using *Pleurotus pulmonarius* at 5WAP.

A mean of 5.80 was obtained for the for number of leaves at 7 WAP in the group treated with the consortia of *Alkaligenes faecalis* (LC349889.1), *Pseudomonas azotoformans* (LC349894.1) and *Bacillus mycoides* (LC349897.1), this is similar to that obtained by Adenipekun *et al.*(2013), in which they observed a range of 4.500-10.666 number of leaves in *C. Olitorius* grown on a *Pleurotus pulmonarius* remediated soil contaminated with oil at 5WAP.

The biggest stem diameter observed among the different treatment groups in this study was found in the group treated with the consortia of *Alkaligenes faecalis* (LC349889.1), *Pseudomonas azotoformans* (LC349894.1) and *Bacillus mycoides* (LC349897.1) having a mean diameter of 1.35 mm. This is comparable to that obtained by Adenipekun *et al.*(2013), in which they observed a range of 0.128-1.05 mm for stem diameter of *C. Olitorius* grown on a *Pleurotus pulmonarius* remediated soil contaminated with oil at 5WAP.

Analysis of the harvested *C. olitorius* plants revealed that for proximate matter such has % crude fibre, ether extract (fat) and fresh weight most of the treatment groups performed well having a range of 18.72-22.62; 1.35-2.00 and 0.22-2.54 respectively compared to values of 20.30; 0.12 and 1.11 respectively obtained in some previous studies (Adenipekun *et al.*, 2013, Ndlovu and Afolayan, 2008; Yekeen *et al.*, 2013). Other proximate matters such as % ash, % crude protein and % moisture had a range of 14.97-24.57; 4.44-15.82 and 43.65-62.00 respectively and these were low compared to values of 21-21.40; 21.12 and 84.28 respectively obtained by Onwordi *et al.* (2009) and Acho *et al.* (2014). High ash content in food is a measure of high deposit of mineral contents (Akpabio *et al*., 2012). The value obtained in this study suggests that the *C. Olitorius* harvested was moderately rich in mineral elements. One major reason why the harvested *C. Olitorius* is not high in fibre content is because the harvested plants were observed to absorb heavy metals and this could have affected its mineral content. Dietary proteins are important for natural synthesis and maintenance of body tissues, enzymes and hormones as well as other substances required for healthy functioning of the body system (Hayat *et al*., 2014). The protein value obtained from this study 4.44±1.33 to 15.82±1.90 suggests that *C. olitorius* can effectively contribute to the daily protein needed. Even though the value is low compared to protein values from other reports, Gqaza *et al.* (2013) however said that any plant food that provides more than 12 % of its caloric value from protein is considered a good source of protein. The moisture content obtained for *C. Olitorius* in this study though low when compared to that obtained by some previous authors, shows that the harvested *C. olitorius* can be easily susceptible to spoilage by micro-organisms during storage (George, 2003).

The analysis of the harvested *C. Olitorius* plant revealed the presence of heavy metals in the leaves; this is an indication that there was an uptake of these heavy metals by the roots of *C. olitorius* and this was followed by its translocation which is in agreement with Peralta-Videa *et al.* (2002). According to earlier studies by Kashem and Singh (2004) and Rieuwerts *et al.* (2006), it was reported that at pH ranges between 4.0-8.5, metal cations are mobile while anions tend to transform to oxide minerals, thus increasing their concentration in the environment, the pH of the treated soil samples were found to be between 8.41 and 8.52, this could be one of the reasons the heavy metals were easily absorbed and translocated in the plant. According to Muhammad *et al.* (2008), leafy vegetables grown in heavy metal contaminated soils, accumulate higher amounts of metals than those grown in uncontaminated soils. This is because they are capable of absorbing these metals through their roots. As earlier reported by Akan *et al.* (2009), vegetables accumulate heavy metals in their edible and non-edible parts as can be seen in the accumulation of heavy

metals in the leaves and stems of *C. Olitorius* in this study. The analysis of the harvested *C. Olitorius* plant revealed that the content of iron (Fe) and zinc (Zn) in the harvested plants was low having a range of 24.95-38.10 and 4.25-17.45 mg/kg respectively compared to the recommended standard of 60 mg/kg (Sanyaolu *et al*., 2011; Ayejuyo *et al*., 2014).

The post bioremediation and post-harvest analysis of the treated soils revealed an increase in the pH, total organic carbon, total nitrogen and available phosphorus of the treated soils. The pH and available phosphorus observed in the treated soil in this study were higher than the pH of 5.1-6.5 and available phosphorus of 20 mg/kg observed in studies by Brady and Weil (2008) and Holland *et al.* (1981) respectively. An increase was observed in the concentration of minerals such as calcium, potassium and sodium of the treated soils. The isolates used in this study were able to achieve high reduction in heavy metal concentration in the soil. For instance, there was 57.99% reduction in the concentration of iron in the group treated with a mixed culture of *Alcaligenes faecalis* (LC349889.1) and *Bacillus mycoides* (LC349897.1), groups treated with *Pseudomonas azotoformans* (LC349894.1) and *Bacillus mycoides* (LC349897.1) and a mixed culture of *Pseudomonas azotoformans* (LC349894.1) and *Bacillus mycoides* (LC349897.1) had a 98.92% reduction on the concentration of lead, groups treated with *Alcaligenes faecalis* (LC349889.1) and a mixed culture of *Alcaligenes faecalis* (LC349889.1) and *Bacillus mycoides* had a 91.39% reduction on the concentration of cobalt, group treated with *Bacillus mycoides* (LC349897.1) had a 97.42% reduction on the concentration of nickel while the group treated with *Pseudomonas azotoformans* (LC349894.1) had a reduction of 99.89% on the concentration of chromium. This is comparable to results from earlier studies. For instance, Chang *et al.* (1997) reported a *Pseudomonas aeruginosa* PU21 (Rip64) strain with a metal uptake efficiency of 80% within 2 days while Roane *et al.*(2001) reported a *Bacillus* strain H9 with a metal uptake efficiency of 36% within 48 hours. Magyarosy *et al.* (2002) also reported a *Pseudomonas* spp with a metal uptake efficiency of 98% within 4 days. Though this isolates did not work as rapid as those earlier cited, this can be attributed to the fact that soils contaminated with heavy metals are poor in nutrients and bacterial diversity which results in impeded rates of remediation (White *et al.*, 2006).

Conclusion

This study has shown that stimulation of bacterial isolates with organic amendments such as cow manure slurry/gomeya has great potentials in bioremediation. It has also been observed that the use of bacterial consortia rather than individual microorganism works more effectively in bioremediation of heavy metals.

Conflict of Interest

The authors declare that there is no conflict of interest.

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ORIGINAL RESEARCH ARTICLE

Food habit and ecological balance of fish species in Osun river, Nigeria

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Abstract

The food habit and ecological balance of fish species in Osun River were investigated for a period of 12 months (November 2017 to October 2018) with monthly sampling of water and fish species in the river. Fish were sampled using monofilament gill nets of mesh size between 25mm and 101mm. A total of 4544 individuals belonging to 19 species and 10 families were captured. They were grouped based on their feeding habits into herbivores, carnivores, and omnivores. Carnivores dominated the River (38.63%) with Cichlids being the most abundant family (95.10%) and *Oreochromis niloticus* the most abundant fish species (14.10%). The Forage - Carnivore ratio (F/C) for the dry, wet and combined season was 0.64, 0.34 and 0.48 respectively. T-test showed significant differences ($P < 0.05$) between all the trophic groupings in relation to seasons. The mean values recorded across the sampling months were Temperature (18.70 ^{o}C ± 2.69), pH (7.10 ± 0.25) and Dissolved Oxygen (3.20 mg/L ± 0.54) while Ammonia (1.20 mg/L ± 0.18) was significantly different ($P < 0.05$) across the months of study. For the sampling points, mean values recorded were Temperature (17.90 ^{o}C ± 0.31), pH (7.20 ± 0.12), Dissolved Oxygen (3.20 mg/L ± 0.27 mg/L) and Ammonia (0.10 mg/L ± 0.21). The abundance of carnivores is not desirable, and it is therefore essential to maintain the ratio of forage and carnivorous fish species in the river for conservation of fish species.

Keywords: *Fish abundance, Feeding habits, Osun River.*

Introduction

Nigeria is a country blessed with diverse water bodies which is the home of diverse fish species. These water bodies have been reported to contribute about 100% to domestic production and 0.48% to the Nigerian Gross Domestic Product (FAO, 2016). Nigeria is said to be the richest country in terms of fish species across the West African coast (Taiwo, 2008); but over the years, the abundance of fish species has been dwindling as a result of urbanization and industrialization, increase in fishing pressures, climatic factors and overfishing (Iyiola and Jenyo-Oni, 2018). These activities are majorly human-driven, and their severity can either lead to a balanced or an imbalance in the ecological relationship of fish species in aquatic aquifers (Ipinmoroti, 2013).

Ecological balance basically describes how ecosystems are structured and the relationship between the fish species and their environment (Nappi, 2016). Ecological changes referred to as disturbances may occur in an ecosystem as a result of various factors such as food availability, fish species size and composition as well as human activities. Such changes may affect a particular niche in an environment causing a shift in the population balance (Arrington, 2015). Preliminary studies on forage carnivore ratio by Swingle (1950) reported a range of 3 – 6 as the most desirable range for F/C ratios of a balanced fish population in an aquatic system, and values between 1 and 3 indicates the fish population is somewhat crowded with carnivorous fish species and this is not desirable because they can eventually reduce the entire fish population due to predation.

Osun River is a prominent river in the Ogun-Osun river basin in South-Western Nigeria; it contributes significantly to fish production within the state and beyond. The River is fed by various river tributaries and reservoirs which empty diverse fish species into it making it rich in fish composition. In most water bodies, non – predatory fish species (herbivores) are usually more abundant than the predatory fish (carnivores and omnivores) and their ratio is used to determine the ecological balance of fish species in a river. Studies by Yem *et al.* (2011); Badejo and Oriyomi (2015); Komolafe *et al.* (2016) and Iyiola and Jenyo-Oni (2018) reported an abundance of fish species in the Rivers but there is limited documented information on the food habit and ecological balance of fish species in the River which is an important factor in the conservation of fish species. This study will therefore distinguish the fish species caught based on their feeding relationship and investigate their balance by determining the F/C ratio. This measure is essential in proposing a holistic approach to regulation of human and fisheries activities for conservation and management purposes.

Materials and Method

Study Area

Osun River is located between coordinates 6° 33' 35" (6.5597°) North and 4° 3' 46.8" (4.063°) east. Its catchment is surrounded by various fishing communities with diverse human activities ranging from domestic to agricultural practices all of which empty directly or indirectly into the river. The river was stratified based on accessibility and logistical characteristics into seven points tagged A – G (Figure 1). Monthly sampling of water and fish species was from each of these points for a period of 12 months (November 2017 to October 2018).

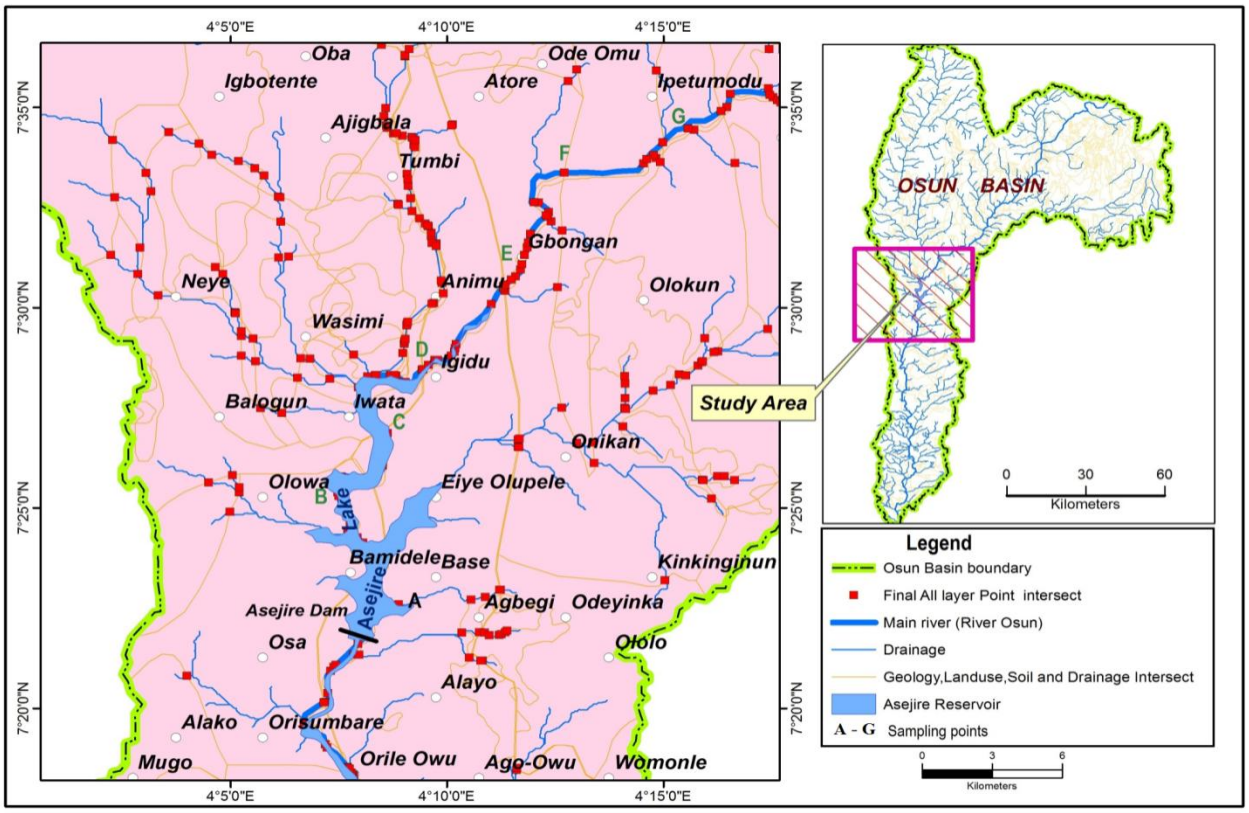

Figure 1: Map of Osun River showing sampling points

Source: Iyiola and Jenyo-Oni (2018).

Assessment of Fish Abundance

Fish species were sampled using a combination of monofilament gill nets with mesh sizes ranging from 25mm to 125mm. These nets were set at the sampling points between the hours of 7.00 pm and 9.00pm and hauled the next morning between 7.00 am and 9.00am. The fish sampled were identified using a combination of monographs by Holden and Reeds (1978) and Olaosebikan and Raji (2013) and their abundance was recorded.

Assessment of Food habit and Ecological balance

The captured fish species were grouped based on their feeding habits into herbivores, carnivores, and omnivores as described by Lewis (1974) and Holden and Reeds (1978). The grouping was done based on the wet and dry season for comparison in feeding habits and a simple food web was illustrated based on the feeding habits. The F/C ratio was calculated using the formula stated by Swingle (1950):

$$\frac{F}{C} = \frac{Herbivores}{Carnivores}$$

Assessment of Water Quality

The samples were collected monthly from the sampling points in 100 ml sterilized bottles between the hours of 7.00 am and 9.00 am. It was measured for Temperature as described by Iyiola *et al.*,(2019), Dissolved Oxygen (DO) using a DO Meter manufactured by Lutron, UK with model number DO-5509 and measured in milligrams per liter (mg/L), pH and Ammonia

using API Freshwater Master Test Kit manufactured by MARS Fishcare, USA (2016) and recorded in (mg/L).

Statistical Analysis

The mean values of data collected on the water quality parameters were separated using Analysis of Variance (ANOVA). Data obtained on fish abundance were analyzed using simple descriptive statistics, percentages and T-test was used to compare the significance among the trophic levels in the wet and dry seasons. Minitab 17.0 was used for all statistical analyses.

Results and Discussion

Fish species abundance and food habit.

A total of 4544 individuals belonging to 19 species were captured during the study. A summary of the feeding habits and fish abundance captured during the study is presented on Tables 1, 2, 3, and 4. In the dry season, a total of 2850 individuals belonging to 8 families were encountered (Table 1). Herbivores had the highest relative abundance (39.30%) with *Oreochromis niloticus* being the highest occurring fish species (15.93%) and the least was *Distichodus rostratus* (0.46%).

Carnivores had a relative abundance of 35.37% with *Alestes baremoze* being the highest occurring fish species (11.93%) and *Hepsetus akawo* and *Schilbeurano scopus* were not captured during this period. The omnivores had the least relative abundance (25.33%) with *Chrysichthys nigrodigitatus* having the highest relative abundance (21.33%) and *Clariasmacromystax, Mormyrups anguilloides* and *Cromeria occidentalis* were not captured during this period. An F/C ratio of 0.64 was calculated during the dry season which indicated that the population of forage and carnivores in the river is not ecologically balanced because it is not within the most desirable ecological range of 3 – 6 (Swingle, 1950). The F/C ratio during this period is an indication of an abundance in carnivores which is not desirable because they will prey naturally on the herbivores thereby causing a shift in balance ratio (Taiwo *et al.,* 2018).

In the wet season, total of 1694 individuals belonging to 10 families were encountered (Table 2). Herbivores had the least relative abundance (25.51%) with *O. Niloticus* having the highest relative abundance of fish species (11.04%) with Red Tilapia and *C. Marie* not captured during the study period. The relative abundance of carnivores was high (36.48%) with *A. Baremoze* having the highest abundance of fish species (13.16%) and *B. Macrolepidotus* was not captured during the study. The abundance of carnivores is an indication of a possible imbalance in population. The omnivores had the highest relative abundance (38.02%) with *C. Nigrodigitatus* being the highest occurring fish species in the group (28.22%) and the least was *Clarias macromystax* with 0.47%. An F/C ratio of 0.34 was calculated during the wet season and was not within the desirable range for a balanced population (3 - 6). From the results, it can be observed at the time of the study that the river was crowded with carnivorous and omnivorous fish species and the entire fish population could be wiped out if measures are not taken regarding the unbalanced state of fish species in the river.

In the whole year (wet and dry season combined) (table 3), carnivores had the highest relative abundance (38.63%) and Alestidae was the most abundant family (29.80%). An F/C ratio of 0.48 was calculated which indicates an unbalanced population of fish species in the river. The

abundance of carnivores is an indication that the population is unbalanced, and its dominance has a negative effect on other fish species in the river.

Table 1: Abundance and trophic grouping of fish species captured during the dry season

Herbivores	Abundance	Abundance (%)	Carnivores	Abundance	Abundance (%)	Omnivores	Abundance	Abundance (%)
Cichlidae*			Schilbeidae*			Mochokidae*		
O. niloticus	454	15.93	*S. mystus*	128	4.49	*S. budgetti*	39	1.37
S. galileausgalileaus	284	9.96	*S. uranoscopus*	0	0	Mormyridae*		
Coptodonzillii	317	11.12	Alestidae*			*Mormyrusrumerume*	75	2.63
Red Tilapia	25	0.88	*H. forskalii*	308	10.81	*M. anguilloides*	0	0
Coptodonmarie	27	0.95	*A. baremoze*	340	11.93	Bagridae *		
Distichontidae*			*B. macrolepidotus*	213	7.47	*C. nigrodigitatus*	608	21.33
D. rostratus	13	0.46	Latidae*			Clariidae*		
			Lates niloticus	19	0.67	*C. macromystax*	0	0
			Hepsetidae*			*C. occidentalis*		0
			H. akawo		0			
Total	1120	39.30		1008	35.37		722	25.33

Forage/Carnivore ratio (F/C) = Herbivores/ Carnivores + Omnivores = 0.64

Total fish species = 2850

Note: Omnivores were grouped with carnivores in F/C estimation as stated by Ipinmoroti (2013), * refers to Family of fish species

Table 2: Abundance and tropic grouping of fish species captured during the wet season

Herbivores	Abundance	Abundance (%)	Carnivores	Abundance	Abundance (%)	Omnivores	Abundance	Abundance (%)
Cichlidae*			**Schilbeidae***			**Mochokidae***		
O. niloticus	187	11.04	*S. mystus*	178	10.51	*S. budgetti*	116	6.85
S. galileausgalileaus	25	1.48	*S. uranoscopus*	5	0.30	**Mormyridae***		
C. zillii	157	9.27	**Alestidae***			*M. rumerume*	9	0.53
Red Tilapia	0	0	H. forskalii	140	8.26	*M. anguilloides*	18	1.06
C. marie	0	0	*A. baremoze*	223	13.16	**Clariidae***		
Distichontidae*			*B. macrolepidotus*	0	0	*C. macromystax*	8	0.47
D. rostratus	63	3.72	**Hepsetidae***			*C. occidentalis*	15	0.89
			H. akawo	54	3.19	**Bagridae***		
			Latidae*			*C. nigrodigitatus*	478	28.22
			L. niloticus	18	1.06			
Total	**432**	**25.51**		**618**	**36.48**		**644**	**38.02**

Forage/Carnivore ratio (F/C) = Herbivores/ Carnivores + Omnivores = 0.34

Total fish species = 1694

Note: Omnivores were grouped with carnivores in F/C estimation as stated by Ipinmoroti (2013), * refers to Family of fish species

Table 3: Abundance and tropic grouping of fish species captured throughout the study period

S/N	Herbivores	Abundance	Abundance (%)	Carnivores	Abundance	Abundance (%)	Omnivores	Abundance	Abundance (%)
1	*O. niloticus*	641	14.10	*S. mystus*	306	6.73	*S. budgetti*	155	3.41
2	*S. galileausgalileaus*	309	6.80	*S. uranoscopus*	5	0.11	*M. rumerume*	84	1.85
3	*C. zillii*	474	10.43	*H. forskalii*	448	12.72	*C. macromystax*	8	0.182
4	Red Tilapia	25	0.55	*H. akawo*	54	1.18	*M. anguilloides*	18	0.40
5	*C. marie*	27	0.59	*L. niloticus*	37	0.81	*C. occidentalis*	15	0.33
6	*D. rostratus*	76	1.67	*A. baremoze*	563	12.39	*C. nigrodigitatus*	1086	21.04
7				*B. macrolepidotus*	213	4.69			
	Total	1552	34.14		1626	38.63		1366	27.21

Forage/Carnivore ratio (F/C) = Herbivores/ Carnivores + Omnivores = 0.48

Total fish species = 4544

Note: Omnivores were grouped with carnivores in F/C estimation as stated by Ipinmoroti (2013), * refers to Family of fish species

The abundance of herbivores, carnivores and omnivores during the study period is presented in tables 4, 5 and 6 respectively. For herbivores, the Cichlids family had the highest relative abundance (95.10%) out of the total 1552 individuals encountered during the study period. The abundance of herbivores in Nigerian freshwaters have been reported by Yem *et al.*, (2011); Ipinmoroti (2013) and Badejo and Oriyomi (2015). Cichlids are highly targeted and are the most common family of fish species in Nigerian freshwaters because they are highly prolific and their availability is all year round (Ogwutu-Ohwayo 2005; Kareem *et al.*, (2015). A total of 1626 individuals were encountered in the carnivore's group with family Alestidae having the highest abundance (75.28%). A total of 1366 individuals belonging to the omnivores group were encountered with family Bagridae having the highest abundance (79.50%). The abundance of herbivores, carnivores and omnivores was higher in the dry season with 72.16%, 61.99% and 52.86% respectively than the wet season with 27.48%, 38.01% and 47.14 respectively. This was expected because of the cease in breeding activities during the dry season in which fish species will be dominate the open waters (Negi and Mamgain, 2013).

Table 4: Abundance by family of Herbivores species captured throughout the study period

Herbivores	Abundance (wet season)	Abundance (dry season)	Total abundance	Relative abundance (%)
Cichlidae	369	1107	1476	95.10
Distichontidae	63	13	76	4.90
Total	432	1120	1552	100
Relative abundance (%)	27.84	72.16		

Table 5: Abundance by family of Carnivores species captured throughout the study period

Carnivores	Abundance (wet season)	Abundance (dry season)	Total abundance	Relative abundance (%)
Schilbeidae	183	128	311	19.13
Alestidae	363	861	1224	75.28
Hepsetidae	54	0	54	3.32
Latidae	18	19	37	2.28
Total	618	1008	1626	100
Relative abundance (%)	38.01	61.99		

Table 6: Abundance by family of Omnivores species captured throughout the study period

Onmivores	Abundance (wet season)	Abundance (dry season)	Total abundance	Relative abundance (%)
Mochokidae	116	39	155	11.35
Mormyridae	27	75	102	7.45
Clariidae	23	0	23	1.68
Bagridae	478	608	1086	79.50
Total	644	722	1366	100
Relative abundance (%)	47.14	52.86		

The wet (0.34), dry (0.64) and combined season (0.48) had an F/C ratio which fell within an unbalanced fish population. The fish abundance was higher in the dry season (2850) than in the wet season (1694), this may be due to the breeding activities during the wet season which initiated migration from the open waters to shallow areas reducing their population whereas, in the dry season, most fish species do not undergo breeding exercises and are populated in the open waters (Negi and Mamgain, 2013). The increase in fish abundance during the dry season resulted in higher F/C ratio though not to sustainable level. The imbalance in the fish community within the ecosystem may be as a result of unregulated fishing activities and the open-access nature of the fisheries in the river which made it possible for the fishermen to target certain species at the expense of the ecosystem. A similar result was observed in Lake Victoria in which fish species disappeared as a result of human activities and interventions (Ogwutu-Ohwayo, 2005). Measures such as the reduction in fishing pressure and establishing open and closed seasons may salvage the fish population (Swingle, 1950).

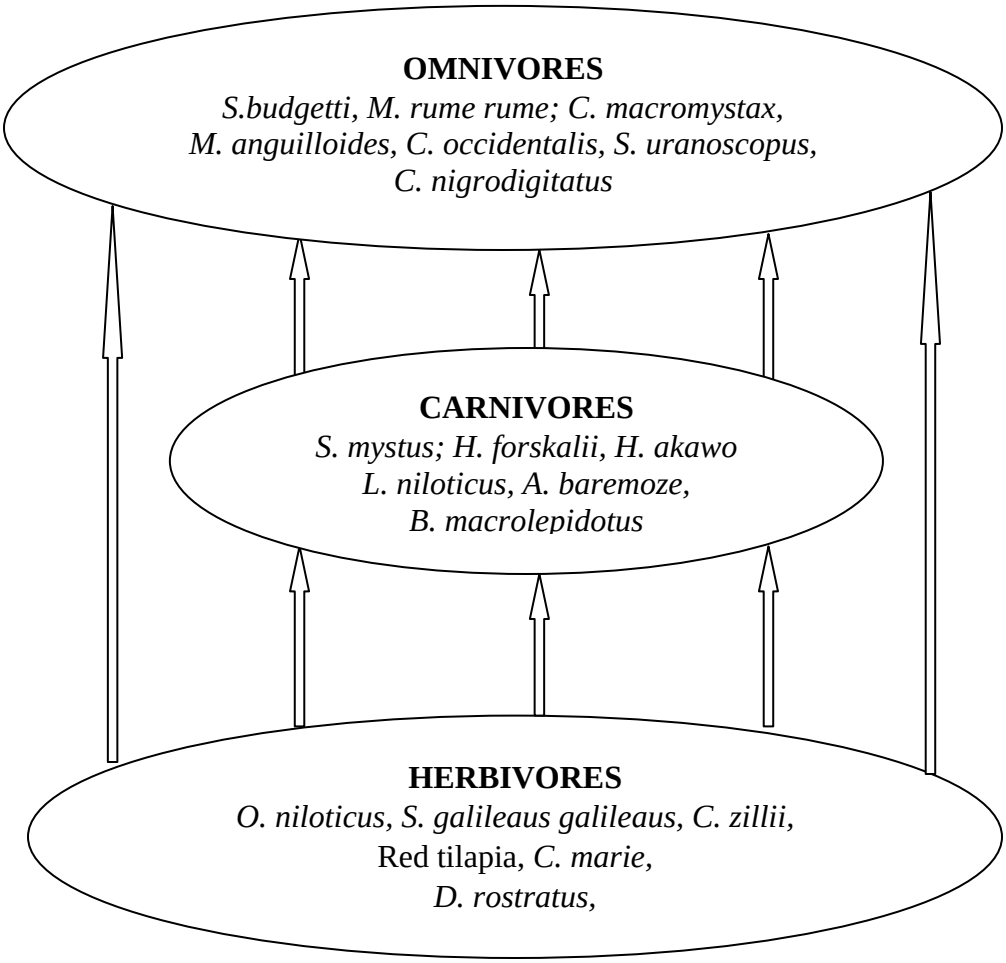

Figure 2: A food web of fish species encountered during the study period

The summary of food habit is presented in Table 7 and it was also observed that the abundance of herbivores was highest in the dry season (39.30%) while carnivores and omnivores were highest in the wet season with 36.48% and 38.02% respectively. Figure 2 illustrates the food web of fish species encountered during the study period. The herbivores are eaten by the carnivores while the omnivores eat both herbivores and carnivores.

Table 7: Summary of food habits observed during the study period

Feeding habit	Dry (%)	Wet (%)	Combined (%)
Herbivores	39.30	25.51	34.14
Carnivores	35.37	36.48	38.63
Omnivores	25.33	38.02	27.21
Forage Carnivore ratio	0.64	0.34	0.48

T-test analysis values showed that there were significant differences ($P < 0.05$) for herbivores (t = 2.124), carnivores (t = 1.481) and omnivores (t = 0.895); this implied that the fish abundance for each trophic group differed with seasons (table 8) and similar results were reported by Taiwo *et al.,* (2018) and Ipinmoroti (2013).

Table 8: T-test analysis on the number of Herbivores, Carnivores and Omnivores during the study period

Group	T – value	Df	Sig. (2-tailed)
HD vs HW	2.124	5	.087*
CD vs CW	1.481	5	.199*
OD vs OW	.895	54	.422*

***** *P<0.05 significance; HD – Herbivores in Dry season; HW – Herbivores in the wet season; CD – Carnivores in Dry season; CW – Carnivores in Wet season; OD – Omnivores in the dry season; OW – Omnivores in the wet season*

Water quality parameters

Water is required for sustenance of life for aquatic organisms and it must be adequate both in quantity and quality. The quality of water discharged into the water system is influenced by the wastes generated by the prevailing anthropogenic activities (Iyiola *et al.*, 2019), the geogenic factors of the water shed and the basement complex. Fish is a cold-blooded animal, and the temperature of the surrounding water dictates the body temperature and the responses of fish to various metabolic processes (Bhatnagar and Devi, 2013).

The mean values of water quality parameters measured across the months and sampling points during the study period are presented in Tables 9 and 10 respectively. Across the sampling months, the highest mean value for temperature was recorded in January (24.0 $^{\circ}$C ± 0.22) while the least was recorded in July (15.1 $^{\circ}$C ± 0.14); the highest mean value was recorded in point A (18.9 $^{\circ}$C ± 0.12) while the least was recorded in point C (17.4 $^{\circ}$C ± 0.11). A steady decrease in values recorded was observed across the months during the wet season from April and later increased in October the values decreased as the rain season progressed. There were fluctuations in recorded values were observed during the dry season (November to March) which may be due to the fluctuating environmental conditions (FAO, 2016). The overall mean monthly value recorded from the river (18.7 $^{\circ}$C ± 2.69) and across the sampling points (17.90 ^{o}C ± 0.31) was below the recommended range of 25 – 32 $^{\circ}$C for the sustenance of aquatic life (Viveen *et al*., 1985). Although some months in the dry season had values within the recommended range while the entire values recorded across the sampling points were below the recommended levels.

DO was highest in November (4.3 mg/L ± 0.05) and least in March (2.6 mg/L ± 0.16) while across the sampling points, DO was highest at points D (3.4 mg/L ± 0.52) and E (3.4 mg/L ± 0.42) and least in points A (3.1 mg/L ± 0.12), B (3.1 mg/L ± 0.01) and F (3.1 mg/L ± 0.31). Mean values fluctuated within the same range during the dry season and slight variations were observed in mean values during the wet season. Across the sampling points, the concentration of DO varied with no significant difference *(P > 0.05)* across the points. The fluctuations in the dry season were expected because temperature has direct effects on DO concentration in water (Bhatnagar and Devi, 2013). The overall mean value across the months (3.2 mg/L ± 0.54) and sampling points (3.20 mg/L ± 0.27) were below the recommended range of 4 mg/L as stated by Boyd and Linchtkoppler (1979).

The mean monthly pH values recorded (7.2 ± 0.12) were within the required range of 6.0 and 8.5 as stated by Boyd and Linchtkoppler (1979) and Parvathi and Sivakumar (2016). The values measured across the months and sampling points were within the range as stipulated except for

August and October which had mean values below the recommended range as stated by Parvathi and Sivakumar (2016). Ammonia level measured varies significantly ($P<0.05$) across the months and the locations. The highest monthly mean value recorded for Ammonia was in July (0.3 mg/L ± 0.09), but the level was below detectable level in the months of November and January. Similarly, the level was below detectable limit in stations E, F and G while the highest mean value was recorded in point B (0.3 mg/L ± 0.11). The high levels of ammonia in the river (1.2 mg/L ± 0.18) depicts that it receives high influx of wastes when compared with the recommended value of < 0.05mg/L (Boyd and Linchtkoppler, 1979).

Table 9: Mean water quality parameters measured across the months during the study period

Season	Parameters	Temperature (°C)	DO (mg/L)	Ammonia (mg/L)	pH
Dry	November	22.60 ± 0.20[a]	4.30 ± 0.05[a]	0.00	7.30 ± 0.03[a]
	December	19.30 ± 0.29[a]	2.60 ± 0.16[a]	0.10 ± 0.05[a]	7.30 ± 0.43[a]
	January	24.00 ± 0.22[a]	3.90 ± 0.03[a]	0.00	7.40 ± 0.10[a]
	February	19.10 ± 0.26[a]	2.90 ± 0.06[a]	0.10 ± 0.05[a]	7.00 ± 0.79[a]
	March	19.30 ± 0.29[a]	2.60 ± 0.16[a]	0.10 ± 0.05[a]	7.00 ± 0.03[a]
Wet	April	19.10 ± 0.26[a]	2.90 ± 0.14[a]	0.10 ± 0.05[a]	7.10 ± 0.05[a]
	May	18.30 ± 0.18[a]	3.50 ± 0.10[a]	0.10 ± 0.05[a]	7.10 ± 0.34[a]
	June	17.00 ± 0.00[a]	3.00 ± 0.30[a]	0.10 ± 0.07[a]	7.20 ± 0.03[a]
	July	15.10 ± 0.14[a]	3.30 ± 0.15[a]	0.30 ± 0.09[c]	7.00 ± 0.08[a]
	August	15.40 ± 0.37[a]	3.10 ± 0.06[a]	0.20 ± 0.10[b]	6.80 ± 0.06[a]
	September	15.90 ± 0.63[a]	3.10 ± 0.06[a]	0.10 ± 0.09[a]	7.00 ± 0.01[a]
	October	18.90 ± 0.51[a]	3.20 ± 0.07[a]	0.20 ± 0.10[b]	6.90 ± 0.07[a]
	Mean	**18.70 ± 2.69**	**3.20 ± 0.54**	**1.20 ± 0.18**	**7.10 ± 0.25**

Values with different superscripts along the same column are significantly different (P < 0.05); ± indicates Standard Error of Mean (SEM)

Table 10: Mean water quality parameters measured across the sampling points during the study

Sampling points	Temperature (^oC)	DO (mg/L)	Ammonia (mg/L)	pH
A	18.9 ± 0.12[a]	3.1 ± 0.12[a]	0.2 ± 0.16[a]	7.3 ± 0.11[a]
B	17.5 ± 0.12[a]	3.1 ± 0.01[a]	0.3 ± 0.11[a]	7.3 ±0.52[a]
C	17.4 ± 0.11[a]	3.3 ± 0.21[a]	0.2 ± 0.12[a]	7.3 ± 0.13[a]
D	17.9 ± 0.03[a]	3.4 ± 0.52[a]	0.2 ± 0.01[a]	7.1 ±0.21[a]
E	18.2 ± 0.15[a]	3.4 ± 0.42[a]	0.0	7.1 ±0.42[a]
F	17.9 ± 0.21[a]	3.1 ± 0.31[a]	0.0	7.1 ± 0.22[a]
G	17.7 ± 0.19[a]	3.2 ± 0.11[a]	0.0	7.2 ± 0.62[a]
Mean total	17.90 ± 0.31	3.20 ± 0.27	0.10 ± 0.21	7.2 ± 0.12

Values with different superscripts along the same column are significantly different (P < 0.05); ± indicates Standard Error of Mean (SEM)

Conclusion and Recommendation

It is evident from the study that the food habit and ecological balance of fish species are influenced by the prevailing seasonal conditions, fishing activities and water quality. It was observed that the river received wastes which were evident by the increased level of ammonia measured from the river. The dry, wet and combined seasons had forage to carnivore ratios below the ideal limit for balanced population. The possible reason for this is the abundance of carnivores in the river and from the ecological view; they pose a threat to the ecosystem. For management purposes, their population may be reduced by fishing through the food web in which trophic levels are simultaneously fished by anglers. Also, it is essential to regulate fishing pressure, establish open and closed seasons and introduce measures such as fishing across the trophic levels for the sustainability of fish species in the River.

Conflict of Interest: The authors declare that there is no conflict of interest

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ORIGINAL RESEARCH ARTICLE

Influence of organic mulch sources and time of their application on the yield of plantain (*Musa spp.*) in Owerri, southeast Nigeria

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ABSTRACT
The effect of five organic mulches (oil palm bunch refuse, oil palm fibre, woodchips, sawdust and multispecies thrash) applied at three different times {at planting, 3 and 6 months after planting (MAP)} at Owerri in the tropical rainforest agro-ecology of southeastern Nigeria were investigated. The experiment was a 3 x 5 factorial experiment laid out in a Randomized Complete Block Design with three replications. Mulching of plantain using oil palm bunch refuse produced highest plantain bunch yield (17.68 t ha^{-1}) while mulching with multispecies thrash gave lowest plantain bunch yield (12.02 t ha^{-1}). Mulching of plantain at 3 MAP produced bigger plantain bunch yield (15.79 t ha^{-1}) than mulching of plantain at planting (14.44 t ha^{-1}) and at 6 MAPS (13.21 t ha^{-1}). Mulching of plantain at 3 MAPS with oil palm bunch refuse stimulated early sucker proliferation, conserved soil moisture content, sustained plantain bunch yield and increased number of fingers per bunch in crop plantain and first ratoon bunch yield. These findings are important for the development of effective mulching strategy for rain fed zones in southeast Nigeria.

Keywords: *Plantain, Organic mulches, Oil palm bunch refuse, Southeast Nigeria*

Introduction
Plantain (*Musa AAB* group) is an important starchy food crop in the humid tropics of south eastern Nigeria. Plantain is a staple food which is eaten either fried, boiled or roasted, and consumed alone or together with other food. Nutrient contents of plantain per 100 gram include fiber (3 g), vitamins A (63 ug), C (23 mg) and B-6 (0.29 mg), magnesium (57 mg), potassium (663 mg) and a poor source of protein (2 g) and fat (0.22 g) and 60 mg of iron in unripe plantain (Cafasso, 2019).

Plantain production is improved by applying appropriate agronomic practices which enhance crop and ratoon plantain bunch yield. Organic mulches have been established for sustainable

plantain production systems in southeast Nigeria because the agro-ecology of southeastern Nigeria is characterized by high annual rainfall (2500 mm) and temperature (20 – 32 °C) (Emma-Okafor *et al.,* 2019) which encourages fast weed development, excessive evaporation and consequently wilting of plantain. Mulching with organic material lowers soil temperature at the root zones, smothers weeds and improves soil physical, chemical and biological properties and crop productivity (Ibeawuchi *et al.,* 2008).

The choice of organic mulching materials is usually determined by local availability at the time of mulching of the specific crop. Southeastern states fall within the oil palm belt of Nigeria and has high accumulation of oil palm waste products such as palm kernel oil, palm kernel shell, oil palm bunch refuse, palm kernel shell and fibres (Law-Ogbomo *et al.,* 2019). Also, Nigeria has about 11 million hectares of forest and about 5.5 million hectares of other wooded land (Akhator et al., 2017). Thus, the Nigerian wood industry is also another contributor of organic wastes such as sawdust and wood chips which are utilized in mulching. Farmers often explore cheaper sources of mulching by using on-farm crop residues and weeds in order the reduce cost of production (Emma-Okafor *et al.,* 2017).

Some investigations (Emma-Okafor *et al.*, 2017; Echezona *et al.*, 2011) have shown that plantain responds positively to organic mulch sources. Mulching plantain using *Mucuna* populations of 20,000 and 10,000 manured with 10-20 tha^{-1} poultry manure produced heaviest plant crop plantain bunches (15-18 kg $plant^{-1}$) (Emma-Okafor *et al.,* 2017). Bunch yield of 4.6 tha^{-1} was reported in plantain mulched with sawdust plus glyphospate treatment (Echezona *et al.,* 2011).
Time of mulching is also an important agronomic practice that ensures enough soil moisture conservation for plantain growth and yield and effective weed control. Applying dry guinea grass to cucumber at 3 weeks after planting produced 10.05 $tha^{-1}$of cucumber (Ibeawuchi *et al.,* 2008). Mulching ginger with 10$tha^{-1}$of green *Chromolaena odorata* at planting produced yield of 26.93 tha^{-1} (Akinwumi *et al.,* 2013). However, there is paucity of information on time of mulching in plantain.

Therefore, this study investigated the effects of three times of mulching plantain with different organic mulch sources on weed suppression, growth and yield of plantain in the tropical rainforest of Southeastern Nigeria.

Materials and Methods

The experiment was carried out during the 2010/2011 early cropping season at the Teaching and Research Farm, Federal University of Technology, Owerri in the Southeastern agro-ecology of Nigeria. The climate is characterized by wet and dry seasons. The rainy season usually begins mid- March and ends in November with a little dry spell (August break) occurring in August. The dry season starts in mid-November and ends in March. The annual rainfall is about 2500 mm and is bimodal with peaks in July and September while temperature ranges between 20 - 32°C. The soil is deep porous ultisol derived from sandy deposits in the coastal plains which are highly weathered (Onweremadu *et al.* 2007). The low natural fertility is usually maintained by bush fallow systems. Land was manually cleared, parked and stumped. The plantain Musa AAB variety Ogoni red was spaced 3.0 x 2.0 m and planted in 60.0 x 60.0 x 60.0 cm holes. The sword suckers from the University plantation orchard were pruned and allowed to dry under shade overnight before planting.

The treatments consisted of five organic mulch sources (oil palm bunch refuse, oil palm fibre, woodchips, sawdust and multispecies thrash) applied at three different times {at planting (April) and 3 MAP (July) and 6 MAP (October)} to plantain. The experiment was a 5 x 3 factorial experiment in a Randomized Complete Block Design with three replications. Wood chips (*Tectona grandis)* and sawdust (*Triplochiton scleroxylon*) were obtained from Owerri timber market. Multispecies thrash (*Panicum maximum* and *Aspilia africana*) were obtained from cleared vegetation from the experimental site and were sundried for two weeks**.** Each planting depth was mulched with 4 t ha^{-1} (2kg $hole^{-1}$) of the different organic mulch sources at planting. A blanket split application of 10 tha^{-1} of cured poultry manure at 2 and 4 MAPS (total of 6 kg $plant^{-1}$) was spread on the organic mulches at planting. Mulch sources were analysed for chemical contents. Percentage soil moisture content was determined in February 2011. Data were also collected on percentage productive mat, crop plantain and ratoon bunch yield and weed dry weight. Data collected were subjected to analysis of variance using Genstat 2011 and the means were separated using Fisher's Least Significant Difference.

Climatic data

Minimum and maximum temperatures were high and stable from April to September 2010; rose in October, peaked in January 2011 and stabilize again in March 2011 (Table I). Rainfall dropped rapidly from November 2010 to zero rain in December 2010 and January 2011. Relative humidity in January and February dropped to the lowest in January and February (68 %).

Table I: The climatic data of Owerri rainforest agro-ecology of Southeastern Nigeria from April 2010 – May 2011

Month	Rainfall (mm)	Average Temperatures (^oC)		Average relative Humidity (%)
		Min.	Max.	
April	122.00	23.70	31.20	75.00
May	240.00	28.50	30.65	76.80
July	286.00	27.50	31.90	74.40
August	305.00	27.80	31.20	74.80
September	330.00	27.50	31.50	74.60
October	398.50	19.00	32.00	75.00
November	108.00	26.00	32.50	78.70
December	0.00	26.50	32.00	79.00
January	0.00	29.50	33.00	68.00
February	40.50	28.50	32.50	68.00
March	122.20	29.00	30.62	73.04
April	156.24	23.70	31.20	75.20
May	248.00	28.50	30.65	76.80

Source: ADP, Imo State

Chemical content of the mulch sources and poultry manure

Percentage nitrogen was highest in oil palm bunch refuse (1.85 %), followed by oil palm fibre (1.14 %) while woodchip (0.14 %) had the least percentage nitrogen content (Table II). Carbon content was highest in woodchip (55.10 %) and least in multispecies thrash (11.28%). Potassium was highest in oil palm bunch refuse (7.4 cmol kg^{-1}) and least in oil palm fibre (3.04 cmol kg^{-1}). Lignin content was highest in woodchip (13.80 %) and least in multispecies thrash (6.34 %).

Chemical analysis of poultry manure showed that poultry manure is alkaline (8.13) and contained 4.49 % of nitrogen, 3.06 % of carbon, and 15.54 cmol kg^{-1} of potassium.

Table II: Chemical properties of organic mulch sources and poultry manure

Chemical contents	Oil palm bunch refuse	Oil palm fibre	Sawdust	Woodchips	Thrash	Poultry manure
pH	N/A	N/A	N/A	N/A	N/A	8.13
Nitrogen (%)	1.85	1.14	0.17	0.14	0.16	4.49
Potassium (cmol kg^{1})	7.40	5.04	5.20	5.16	3.28	15.54
Phosphorus (cmolkg^{-1})	5.75	3.98	10.18	10.06	0.21	4.78
Calcium (cmolkg^{-1})	2.00	1.87	1.92	1.83	1.30	36.21
Magnesium (cmolkg^{-1})	3.10	2.54	1.63	1.50	1.68	6.82
Lignin (%)	14.10	11.15	12.50	13.80	6.34	0.54
Carbon (%)	55.10	31.20	51.20	55.60	11.28	3.06

NA = Not available

Results

Percentage productive root mat (%)

Mulching of plantain with oil palm fibre produced highest percentage productive root mat (92.96 %) while mulching of plantain with multispecies thrash produced lowest percentage productive root mat (72.20 %). Mulching of plantain at 6 MAPS gave highest percentage productive root mat (90.20 %) while mulching of plantain at planting gave lowest percentage productive root mat (83.42 %). Plantain mulched with oil palm bunch refuse at 6 MAP had highest percentage productive root mat (96.08 %) while plantain mulched with multispecies thrash at 6 MAPS had the least percentage productive root mat (69.60 %) (Table III).

Percentage soil moisture content (%)

Percentage soil moisture content was highest in plots mulched with woodchips (10.71 %) while percentage soil moisture content was lowest in plots mulched with multispecies thrash (7.48 %). There is no significant difference between percentage soil moisture content of plots mulched with oil palm bunch refuse (10.53 %) and woodchips. Mulching of plantain at planting conserved higher soil moisture than mulching of plantain at 6 MAPS (7.93 %). Mulching of plantain at 3 MAPS (8.96 %) gave significantly higher percentage soil moisture than mulching of plantain at 6 MAPS. Percentage soil moisture content was highest in plots mulched with woodchips (10.71%). The interaction effect of organic mulch sources and time of mulching did not significantly affect the percentage soil moisture content.

Yield and yield components

Plantain mulched with oil palm bunch refuse at 3 MAP produced highest crop plantain bunch yield (18.88 t ha^{-1}) while plantain mulched with sawdust at 6 MAP produced least crop plantain bunch yield (10.62 t ha^{-1}) (Table IV). Plantain mulched with oil palm bunch refuse produced highest number of fingers in the crop plantain (39.94) while plantain mulched with multispecies thrash produced least number of fingers in the crop plantain bunch yield (33.19). Time of mulching and the interaction between organic mulch sources and time of mulching did not

significantly affect the number of crop plantain fingers. Plantain mulched with oil palm bunch refuse at 6 MAP produced highest ratoon plantain bunch yield (16.65 t ha^{-1}) while plantain mulched with woodchips at 3 MAP produced least crop plantain bunch yield (8.45 t ha^{-1}) (Table V). Time of mulching and the interaction effect in ratoon bunch yield followed similar trend as in plantain crop yield.

Weed dry weight (t ha^{-1})

Weed dry weight was highest in plot mulched with multispecies thrash (3.32 t ha^{-1}) and lowest in plantain plot mulched with woodchip (1.71 t ha^{-1}). There was no significant difference among plots mulched with oil palm bunch refuse (1.75 t ha^{-1}), oil palm fibre (1.79 t ha^{-1}), woodchip (1.71 t ha^{-1}) and sawdust (1.77 t ha^{-1}). Mulching of plantain at planting produced least weed dry weight (1.72 t ha^{-1}) while mulching at 6 MAPS produced highest weed dry weight (2.65 t ha^{-1}). There was no significant difference between mulching of plantain at planting (1.72 t ha^{-1}) and at 3 MAPS (1.83 t ha^{-1}). There was no significant difference in the interaction effect of organic mulch sources and time of mulching on weed dry weight (Table V).

Table III: Effect of organic mulch sources and time of mulching on percentage productive root mats (%) and Percentage soil moisture content (%) at 3 MAPS

	Percentage productive root mats (%) Time of mulching				Percentage soil moisture content (%) (3 MAP) Time of mulching			
Organic mulch sources	**April (At planting)**	**July (3 MAP)**	**October (6 MAP)**	**Mean**	**April (At planting)**	**July (3 MAP)**	**October (6 MAP)**	**Mean**
Oil palm bunch refuse	86.05	95.39	96.08	92.51	12.17	10.30	9.13	10.53
Oil palm fibre	87.66	94.63	96.59	92.96	9.50	7.87	6.73	8.03
Wood chips	84.29	92.01	93.95	90.09	12.31	10.33	9.50	10.71
Sawdust	84.46	93.67	94.75	90.96	10.26	9.03	7.97	9.09
Multispecies thrash	74.65	72.31	69.65	72.20	8.88	7.27	6.33	7.49
Mean	83.42	89.60	90.20		10.62	8.96	7.93	
$LSD_{(0.05)}$ Organic mulch sources	0.69				0.42			
$LSD_{(0.05)}$ Time of mulching	0.88				0.32			
$LSD_{(0.05)}$ Organic mulch sources x Time of mulching	1.53				NS			

Table IV: Effect of organic mulch sources and time of mulching on crop plantain bunch yield (t ha^{-1}) and number of crop Plantain fingers

Organic mulch sources	Crop plantain bunch yield (t ha^{-1}) Time of mulching				Number of crop plantain fingers Time of mulching			
	April (At planting)	July (3 MAP)	October (6 MAP)	Mean	April (At planting)	July (3 MAP)	October (6 MAP)	Mean
Oil palm bunch refuse	18.52	18.88	16.61	17.68	38.82	42.24	38.46	39.94
Oil palm fibre	18.48	18.82	15.42	17.58	38.78	40.58	36.66	38.67
Wood chips	12.63	14.32	10.52	12.49	34.52	38.64	32.68	35.28
Sawdust	12.84	14.42	10.62	12.63	33.68	36.82	34.28	34.93
Multispecies thrash	10.67	12.52	12.86	12.02	30.56	36.54	32.46	33.19
Mean	14.44	15.79	13.21		35.27	38.96	34.91	
LSD $_{(0.05)}$ Organic mulch sources	0.34				4.64			
LSD $_{(0.05)}$ Time of mulching	0.44				NS			
LSD $_{(0.05)}$ Organic mulch sources x Time of mulching	0.78				NS			

Table V: Effect of organic mulch sources and time of mulching on ratoon bunch yield (tha^{-1}) and weed dry weight ($t\ ha^{1}$)

Organic mulch sources	Ratoon bunch yield ($t\ ha^{1}$) Time of mulching				Weed dry weight ($t\ ha^{1}$) Time of mulching			
	April (At planting)	**July (3 MAP)**	**October (6 MAP)**	**Mean**	**April (At planting)**	**July (3 MAP)**	**October (6 MAP)**	**Mean**
Oil palm bunch refuse	14.07	15.88	16.65	15.53	1.48	1.62	2.16	1.75
Oil palm fibre	14.22	16.05	16.45	15.57	1.54	1.60	2.24	1.79
Wood chips	10.86	12.59	10.57	11.34	1.42	1.54	2.16	1.71
Sawdust	10.14	12.66	12.05	11.62	1.54	1.56	2.20	1.77
Multispecies thrash	8.45	10.28	10.67	9.80	2.64	2.82	4.50	3.32
Mean	11.55	13.49	13.28		1.72	1.83	2.65	
$LSD_{(0.05)}$ Organic mulch sources	**0.04**				**0.28**			
$LSD_{(0.05)}$ Time of mulching	**0.03**				**0.52**			
$LSD_{(0.05)}$ Organic mulch sources x Time of mulching	**0.07**				**NS**			

Discussion

Mulching with organic materials promoted high number of productive root mats. This could be as a result of the addition of organic matter by the organic mulches which stimulated root development, sucker and mat production (Alagba *et al.,* 2017). Nitrogen and phosphorus contents of oil palm bunch refuse, oil palm fibre, sawdust and wood chips were higher than that of multispecies thrash. Plantain mulched with multispecies thrash had lower productive mat and this could be as a result of poor nutrient content of multispecies thrash.

Percentage soil moisture content was sustained by oil palm bunch refuse, sawdust and wood chips. The lignin content of oil palm bunch refuse, sawdust and wood chips probably reduced evaporation from the soil in comparison with oil palm fibre and multispecies thrash. Mulching helps to conserve soil moisture by reducing evaporation, soil water losses and erosion through soil surface and makes moisture available especially during the dry season (Staufer, 2012; Emma-Okafor *et al.,* 2016). Mulching of plantain at planting promoted soil moisture conservation than mulching of plantain at 3 MAP and at 6 MAPS. Early mulching increased percentage soil moisture while delaying mulching till 6 MAP decreased percentage soil moisture. This could be attributed to the exposure of soil surfaces to evaporation and other forms of water losses (Ibeawuchi *et al.,* 2015). Percentage soil moisture was low in plantain mulched at 6 MAPS because rainfall volume had declined at the time of mulching and soil surface was bare since planting and thus, less soil moisture was conserved.

Plantain bunch yield was improved by the application of mulch at planting and at 3 MAPS. Organic mulches are rich in organic matter, and thus supply necessary nutrients for the growth and yield of plantain. Higher yield in plantain mulched with oil palm bunch refuse could be attributed to higher N and K in oil palm bunch refuse (Ibeawuchi *et al.,* 2015; Alagba *et al.,* 2017). Bunch yield of plantain mulched with multispecies thrash was lowest in crop plantain and first ratoon and this could be as a result of low nutrient content of multispecies thrash and the un-replenished nutrient depletion throughout the plantain growth and reproductive phase (Obiefuna, 1991). Mulching of plantain at planting and 3 MAPS resulted in improved soil moisture conservation, organic matter utilization by plantain, crop plantain and ratoon bunch yield.

Weed growth in the plantain plots was suppressed by mulching using different organic mulches. The effectiveness of organic mulch on weed control was highest in plantain mulched at planting using oil palm bunch refuse and woodchips. The effectiveness of oil palm bunch refuse and woodchips mulches in smothering weeds could be as a result of their higher lignin contents and slow rate of decomposition of the mulches. Higher weed dry weight in plantain mulched with multispecies thrash could be as a result of lower lignin content of multispecies thrash which encouraged fast decay and thus could not smother weeds. Weeds compete with plantain for growth resources, space and retard growth and yield in plantain (Emma-Okafor *et al.,* 2017).

Conclusion

Mulching is a pre-requisite agronomic practice in sustainable plantain production in the tropical rainforest agroecology. Organic mulch sources are biologically diverse in nutrient and lignin contents. The type of organic much and time of mulching become of essence in the tropical rainforest characterized by heavy rainfall and high temperature patterns. The efficiency of multispecies thrash may be improved by delayed or continuous applications as in-home gardens

for plantain production. Oil palm bunch refuse and oil palm fibre are ideal for sustainable plantain production irrespective of time of mulching. Woodchips and sawdust are effective for weed suppression.

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ORIGINAL RESEARCH ARTICLE

Novel tier reclassification architecture for non–terrestrial data centre systems

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Abstract
Data centres play an important role in hosting and enabling content access in wireless communication networks and computing applications. The use of data centres is accompanied with high operational costs due to the necessity of powering and cooling. In addition, data centres are mostly terrestrial facilities with different performance levels influenced by the data centre tier rating. The need to reduce operational costs has prompted the siting of data centres in non–terrestrial locations such as the ocean and stratosphere with free cooling benefits. The location of future data centres raises new challenges such as reclassifying data centre Tiers. The Tier re-classification challenge should be addressed to identify factors that influence the performance of non-terrestrial data centres. This paper addresses the challenge of designing tier classification architecture for non-terrestrial data centre systems. In this paper, primary and secondary criteria required for the Tier classification of ocean and stratosphere data centres are identified. The proposed tier re–classification architecture is also compared with the Tier 5 data centre standard recently proposed for terrestrial data centres.

Keywords: *Future Computing, Networking, Data Centres, Non–Terrestrial Locations, Tier Re–Classification*

Introduction

Data centres play an important role in cloud computing and future networks. They have different performance levels in terms of availability (fraction of period in a year where the data centre is expected to be function) and support for redundancy. The performance level of a data centre facility is described by the data centre's tier rating. Data centres are classified into different Tiers i.e. Tier I, Tier II, Tier III and Tier IV (Rytoft, 2013; Balodis et al. 2012, Chen et al, 2016, Uptime Institute, 2018). The requirements considered in each Tier classification are system component redundancy, number of distribution paths, compartmentalization, fault tolerance and concurrent maintainability (Turner et al., 2008). Some of the additional parameters that are also used to determine data centre performance are power usage effectiveness (PUE) (Ayanoglu, 2019), water usage effectiveness (WUE) (Penaherrera, 2018) and water footprint (WF) (Evans et al, 2016). The WUE and WF derive their importance from the reliance of data centres on water for cooling. The aforementioned criteria used in the existing data centre Tier classification applies only to terrestrial data centres.

The necessity of cooling increases data centre operational costs. This has motivated data centre operators to seek solutions to reduce data centre operational costs and enhance the PUE. An approach from Google using the Google DeepMind artificial intelligence solution is described in (Evans et al, 2016). Data centre operational costs can also be reduced by siting data centres in non–terrestrial locations such as the ocean (Periola et al, 2020, Cutlers et al, 2017, Periola, 2019) and the stratosphere (Periola, 2019). Ocean data centres benefit from the low temperature available at the ocean's depths. In a similar manner, stratospheric cooling is beneficial to the cooling of stratosphere data centres.

The siting of data centres in the ocean and stratosphere pose new challenges to the environment because they directly interact with the environment and influence biological conservation. This is because the ocean and stratosphere cannot be easily partitioned like the terrestrial domain. Besides the biological conservation challenge, ocean and stratosphere data centres are affected by different physical threats such aerial and underwater missiles. These factors necessitate re–classifying data centre tiers arising from a change in the geographical location. A change in the geographical location necessitates considering environmental sustainability concerns. The inclusion of environmental sustainability necessitates re-designing data centre Tier classification. The reclassification of Tiers for data centres sited in non-terrestrial locations enables the identification of suitable infrastructure, enabling energy and communication technologies, security and environmental concerns. These factors influence the functioning of non–terrestrial data centres and are identified for stratosphere and ocean-based data centres. This paper addresses the challenge of classifying Tiers for ocean and stratosphere data centres. The challenge is addressed by discussing the performance concerns (alongside identifying the required technologies) for data centres in the ocean and the stratosphere.

The contributions of this paper are described as follows:

1) The paper proposes tier classification architecture for non–terrestrial data centres. The proposed classification architecture uses criteria in two categories. The first category is the primary criteria that focus on the technological specifications required for the realization, ensuring the continued functioning of data centres at a given non–terrestrial geographical location. The concern of ensuring addressing environmental sustainability and preserving biodiversity at a given non–terrestrial location is also a primary criterion. The secondary criteria focus on how non–terrestrial data centres interact with existing applications at a given non–terrestrial location, potential applications and required supporting technologies for data centre located in a given non–terrestrial location.

2) Second, the paper identifies and discusses the role of primary and secondary criteria for stratosphere-based data centres. In this case, the primary criteria are stratosphere altitude and location, aerial vehicle classification, physical security, influence on avian habitat and supported communication capability. The secondary criteria are number of existing aerial applications, aviation policy, number of service providers, and support for open computing, computing payload technology and operational stratosphere temperature.

3) Third, the paper identifies and discusses the role of primary and secondary criteria for ocean-based data centres. The primary criteria are location i.e. ocean zone, vessel category,

number of computing platform service providers, electricity and power consumption, marine conservation interests, and security. The secondary tier classification criteria for ocean-based data centres are computing payload architecture, computing payload update frequency, type of computing payload, number of accessible underwater optic cables, and support for compute intensive deep learning workload.

4) In addition, the discussion in the paper describes how different values of the factors considered in the primary and secondary criteria are combined to give rise to the emergence of novel Tier classification names in the proposed non – terrestrial data centre Tier classification architecture.

This paper is divided into three parts. Sections 2 and 3 discusses data centre Tier re–classification for ocean and stratosphere-based data centres, respectively. Section 4 is the conclusion.

1. Tier Re–Classification of Ocean Based Data Centres

The ocean-based data centre is sited in an environment where marine biodiversity conservation is important. An ocean-based data centre can be located in different ocean regions depending on the ocean's depths. The ocean's regions are the (1) Epipelagic, (2) Mesopelagic, (3) Bathypelagic, and (4) Abyssopelagic zones. The support for scalability should also be considered in underwater data centre Tier classification. Additional factors that should be considered include data centre upgrade costs, vessel category (required for inserting and removing the underwater data centre from the ocean), size of data centre crew, and number of cloud service providers owning the data centre payload. Furthermore, the source of power for operating the underwater data is considered to be the grid, renewable or hybrid. Grid-based underwater data centres derive their operational electricity from the grid as seen in the Phase 2 of Microsoft Project Natick tested at Orkney Island, UK (Roach, 2018). Renewable energy based underwater data centres utilize tidal or wave or marine energy for operation. These underwater data centres have onboard wave energy generators. Hybrid underwater data centres utilize electricity from the grid and renewable sources.

In addition, the performance of underwater data centres should consider the role of additional parameters such as system component redundancy, fault tolerance, concurrent maintainability, number of distribution paths and compartmentalization. It is also necessary to determine the re-usability of some performance factors and metrics that have been previously defined and found to be suitable for terrestrial data centres. Nevertheless, a peculiar factor such as the unique design of the suitable artificial reef that supports computing payload arises due to the use of underwater technology and should be recognized. The approach of a uniquely design artificial reef is considered for two reasons. First, it allows cloud service providers to develop artificial reefs that suit their preferences. Second, artificial reef data centre standards are yet to be defined.

The Tier classification of underwater data centres considers the following factors (arising from the use of the underwater environment).

Location: These are the Epipelagic, Mesopelagic and Bathypelagic and Abyssopelagic zones.

Vessel Category: This describes the class of vessel required to execute all the procedures related to underwater data centres. The vessel or ship classification is done utilizing standards such as that provided by the American Bureau of Shipping (Flis, 2016). The American Bureau of shipping provides information suitable for classifying marine vessels. This can be seen in the marine vessel and mobile offshore unit rules. Another example of an existing standard is the UK's Merchant ship classification and certification (Maritime and Coastguard Agency, 2018). Research work on marine vessel classification can be found in (Gol et al, 2014).

Number of Computing Platform Service Providers: An underwater data centre can host servers belonging to different computing platform service providers. The number of computing service providers that collectively own the servers aboard the underwater data centre indicates the complexity associated with handling and executing tasks related to the underwater data centre.

Electricity and Power Consumption: The data centre can be deemed grid, renewable or hybrid.

Marine Conservation Interests: In the consideration here, underwater data centres are considered to have varying levels of influence with regard to ensuring marine conservation. Three levels of influence i.e. threat levels have been considered. Each of these threat levels describes the ability of underwater data centre to potentially harm marine life at a given underwater location. The threat levels are least threats, medium threats and high threats. Underwater data centres with least threats are sited in ocean regions where their operation affects the smallest number of marine animals and mammals. This can arise when the underwater location has the smallest population of species (marine life and coral reefs). The underwater data centre has a medium threat when sited in regions where the number of marine animals and mammals are considerable. Medium threat underwater data centres are sited in regions with more marine species than least threat underwater data centres. The high threat underwater data centre is one that is sited in an underwater region with a considerably high number of marine animals and mammals.

Security: Underwater data centres have more physical exposure than terrestrial data centres due to the existence of applications in the ocean. Technological advancements make underwater data centres susceptible to threats from underwater missiles with advanced technology. This is because of the increasing sophistication of underwater missile technology (Karako et al, 2017; NMHB, 2020; Woolf, 2020). From the perspective of the threat potential, underwater data centres are classified as low missile threats (LMTs) and high missile threats (HMTs). LMTs and HMTs are sited in regions belonging to developing nations and developed nations, respectively.
The factors of location, vessel category, number of computing platform service providers, electricity and power consumption, marine conservation interests and security are the primary criteria for Tier classification of an underwater data centre.

The secondary criteria for the Tier classification of underwater data centres are design and operational factors that influence the performance of the underwater data centre from a computing and networking perspective. The secondary criteria are:

(1) Computing Payload Architecture – This describes the design architecture of the computing payload used in the servers aboard the underwater data centre. The design architecture can be dis-aggregated or non–disaggregated.

(2) Computing payload update frequency – The upgrade frequency describes the number of epochs within a given period where the underwater data centre's payload is updated i.e. changed to one of improved technology.

(3) Type of computing payload – The type of computing payload describes the underlying data access and computing architecture used in the computing boards of servers in the underwater data centre. Type of computing payload can be either neuromorphic or non-neuromorphic.

(4) Number of accessible underwater optic cables – The number of accessible underwater optical cables describes the number of locations with access to the underwater data centre. In addition, it indicates the deliverable quality of service (QoS) by the underwater data centre to a given location.

(5) Support for compute intensive deep learning workload – The inclusion of this criterion has been deemed necessary due to the emergence of big data processing and deep learning applications. This criterion indicates the ability of the underwater data centre to support deep learning (big data driven application). In this case, the value can be either a yes or no.

The relation between an underwater data centre's primary and secondary criteria is shown in Figure 1. In Figure 1, the information on data centre availability has been obtained from (Turner et al, 2008). The Tier classification of the underwater data centres reuses some metrics from terrestrial data centre Tier classification. The underwater data centre Tier I classification has the following sub–tiers:

Tier 1a grid: The Tier 1a grid underwater data centre has the similar availability and redundancy with Tier 1 terrestrial data centre. The Tier 1a underwater data centre has the least threat to marine conservation and uses grid-based electricity.

Tier 1a renewable: The Tier 1a renewable is similar to the Tier 1a grid underwater data centre but uses renewable energy being incorporated with marine wave and tidal generators. The onboard generators convert ocean wave energy into electricity used to operate the data centre.

Tier 1a Hybrid: This is similar to the Tier 1a grid and Tier 1a renewable except that it uses a combination of electricity obtained from the grid and renewable energy sources.

The underwater data centre has a Tier 2 classification which is similar to Tier 2 terrestrial data centres. The Tier 2 classification of underwater data centres has the Tier classifications of Tier 2a grid, Tier 2a renewable and Tier 2a hybrid. The Tier 2a grid, Tier 2a renewable and Tier 2a hybrid underwater data centre are similar to the Tier 1a grid, Tier 1a renewable and Tier 1a hybrid underwater data centre, respectively. However, Tier 2 underwater data centres have different performance availability and redundancy support from Tier 1 underwater data centres for all sub – classifications described by the suffix of the energy source.

In addition, underwater data centres with a Tier 3 classification are similar to Tier 3 terrestrial data centres. The Tier 3 classification of underwater data centres has Tier classifications of Tier 3a grid, Tier 3a renewable and Tier 3a hybrid. The significant difference between the Tier 3 underwater data centre and Tier 1 underwater data centre lies in the expected performance availability and redundancy. The Tier 3 underwater data centre has a higher expected performance availability and redundancy than a Tier 2 and Tier 1 underwater data centre. This is true for all sub – classifications for a given energy source suffix.

A similar classification pattern holds true for the Tier 4a grid, Tier 4a renewable and Tier 4a hybrid. The Tier 1b grid, Tier 1b renewable and Tier 1b hybrid underwater data centres are similar to the Tier 1a grid, Tier 1a renewable and Tier 1a hybrid underwater data centres; with the difference being that they pose medium threats to marine conservation interests.

A similar classification pattern is applicable to Tier 2b grid, Tier 2b renewable and Tier 2b hybrid underwater data centres. The Tier 3b grid, Tier 3b renewable and Tier 3b hybrid underwater data centres are similar to the Tier 3a grid, Tier 3a renewable and Tier 3a hybrid underwater data centre. However the Tier 3b grid, Tier 3b renewable and Tier 3b hybrid underwater data centres pose medium threat to marine conservation in comparison to the Tier 3a grid, Tier 3a renewable and Tier 3a hybrid underwater data centre that pose the least threats to marine conservation.

In the underwater data centre Tier classification, alphabets with an ascending order indicate increasing threats to the realization of marine conservation. For example, the Tier 1a grid underwater data centre has similar availability and redundancy with a Tier 1b grid underwater data centre. However, the latter data centre (i.e. Tier 1b grid underwater data centre) poses a higher threat to marine conservation threats than the former (i.e. Tier 1a grid underwater data centre). In the proposed classification architecture, the underwater data centre Tier 1a grid is deemed to pose the least threat to marine conservation while the underwater data centre Tier 1b grid poses medium threat to marine conservation. An underwater data centre given as Tier 1c grid is considered to pose the highest threats to marine conservation.

Furthermore, the information on the values of the primary and secondary criteria can be accessed from the metadata of the concerned underwater data centre. The values of primary criteria such as vessel class and location can be accessed from the primary metadata. In a similar manner, the values of secondary criteria such as computing payload architecture and support for compute intensive deep learning workload can be accessed from the underwater data centre’s secondary metadata.

A comparison of the different Tiers for underwater data centres is shown in Table I. In Table I, criteria whose parameters can have a wide range of values without loss of performance are specified as variable.

Table I: Criteria values and comparison for Tier 1 classifications for an underwater data centre.

Metric	Tier 1a Grid	Tier 1a-Renewable	Tier 1a Hybrid	Tier 1b Grid	Tier1b Renewable	Tier 1b Hybrid
Grid	Yes	No	Yes	Yes	No	Yes
Renewable	No	Yes	Yes	No	Yes	No
Hybrid	No	No	Yes	No	No	Yes
Conservation threat	Mild	Mild	Mild	Moderate	Moderate	Moderate
Location	Variable	Variable	Variable	Variable	Variable	Variable
Number of Computing Platform Service Providers	Variable	Variable	Variable	Variable	Variable	Variable
Vessel Category	Variable	Variable	Variable	Variable	Variable	Variable
Security and Threat Arising from Physical Exposure	Variable	Variable	Variable	Variable	Variable	Variable
Value of secondary criteria parameters	Variable	Variable	Variable	Variable	Variable	Variable

2. Stratosphere Based Data Centres – Tier Re – Classification

The Tier re–classification of stratosphere-based data centres is done in a similar manner to that of the ocean-based data centres. In addition to availability and redundancy configuration, the primary criteria of classifying stratosphere-based data centres are:

Stratospheric Altitude and Location- The stratosphere has three regions i.e. the lower stratosphere, middle stratosphere and upper stratosphere. The altitude associated with the lower stratosphere can be defined to be in the range 8 km–18 km (Xian et al, 2019). The altitude of the middle stratosphere is in the range of about 20 km to 40 km(Langematz et al, 2019). The upper

stratosphere's altitude can be considered to lie in the range 40 km to 50 km. This is because the stratosphere's maximum altitude is about 50km (Cervany, 2005).

Aerial Vehicle Classification- This is the aviation classification information for the type of aerial vehicle used in realizing the high-altitude platform that serves as the stratosphere data centre. Different types of aerial vehicles such as balloons, airplanes and airships can be used. The airplanes have categories such as A, B1, C, B2, B2L, B3 and L [22]. The L category has subcategories L1C and L1, L2C and L2, L3H and L3G, L4H and L4G and L5. The classification in (European Union Aviation Safety Agency, 2014) considers helicopters, complex motor–powered aircraft, sailplanes, powered sailplanes, balloons and airships.

Communication Capability- The stratosphere-based data centre is expected to communicate with organizations intending to upload and download data. This is essential to derive value from the stratosphere data centre. In this regard, advances in cognitive radio play an important role in classifying stratosphere data centres. The types of stratosphere data centres from the perspective of communications capability are cognitive radio-based stratosphere-based data centres (CRSDC) and non-cognitive radio-based stratosphere-based data centres (NCRSDC). The CRSDC and NCRSDC are capable and incapable of parameter adaptation and dynamic spectrum access, respectively.

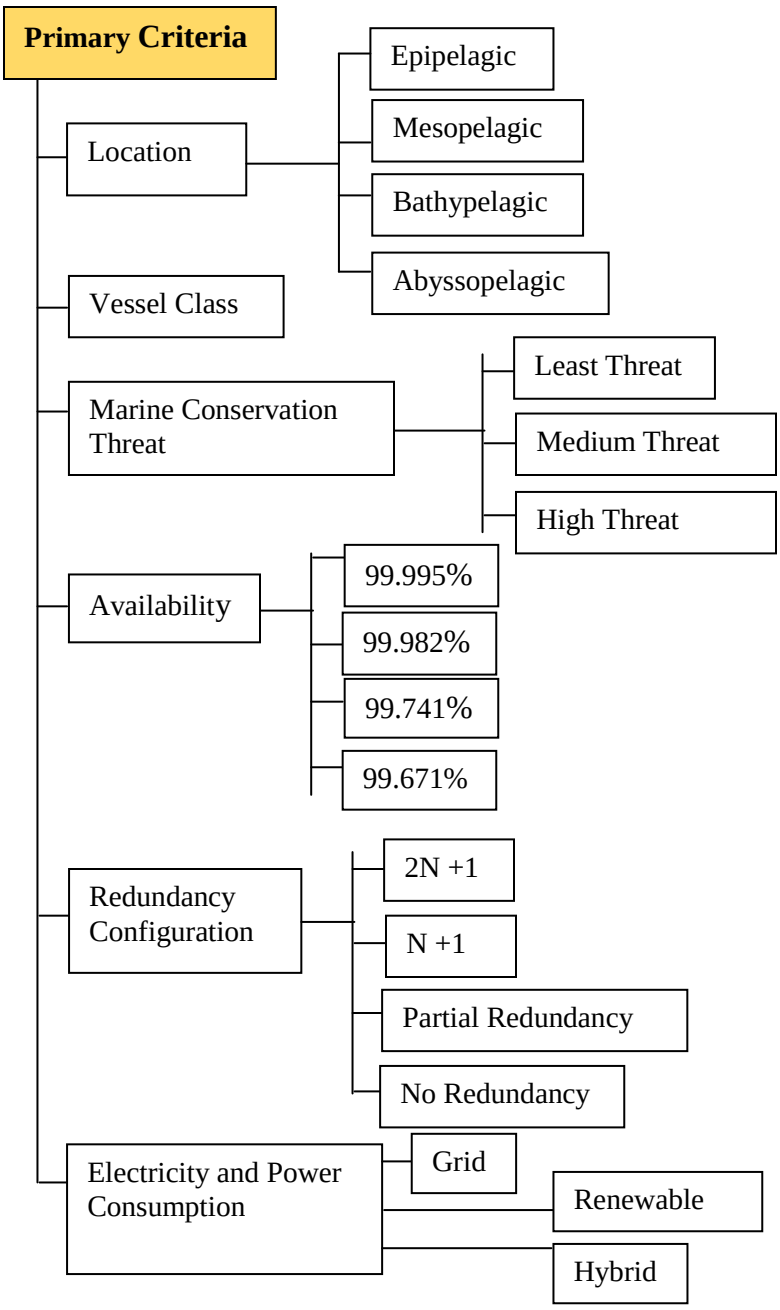

Figure 1: - Organization of Primary Criteria for Tier – Classification of Underwater Data Centres

Physical Security- The stratosphere-based data centre's security is also deemed a primary criterion. Stratosphere data centres can be sited in regions with varying levels of aerial missile activity from malicious entities. From this perspective, the stratosphere-based data centres can be classified as low security, medium security and high security. Low security stratosphere data centres are those with poor payload components to mitigate against aerial threats and co–exist with other applications in the stratosphere. They have a low resilience against stratospheric turbulence. Medium security stratosphere data centres host more advanced security payload than

low security stratosphere data centres. They have a better resilience to stratospheric turbulence than low security stratospheric data centres. High security stratosphere data centres incorporate the most advanced security payload and have the best resilience against stratospheric turbulence. The realization of low security stratosphere data centres, medium security stratosphere data centres and high security data centres are feasible due to advances in radar technology (Greco et al, 2019).

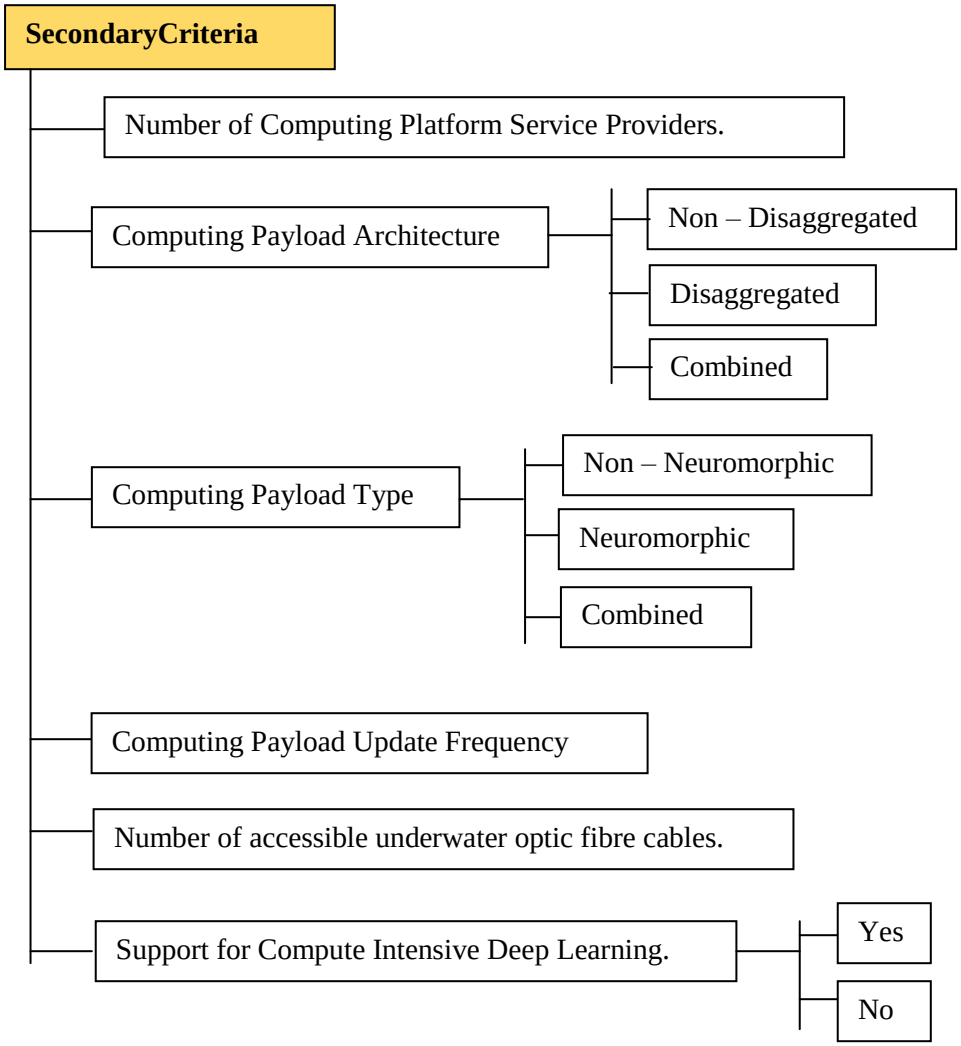

Figure 2:- Organization of Secondary Criteria for Tier–Classification of Underwater Data Centres.

Radar systems have evolved from using non–dynamic spectrum access to dynamic spectrum access technologies. Conventional radars have evolved to incorporating phase scanning arrays (Wiesback et al, 2015). Future radar systems also benefit from multiple input and multiple output technologies, digital beamforming, array imaging and intelligent signal coding (Wiesback et al, 2015). In addition, improved radar system technology can be realized via the incorporation of artificial neural networks (Wan et al, 2019; Wang et al, 2019). High security stratosphere data centres incorporate artificial intelligence driven electronic radar systems. Medium security stratosphere-based data centres incorporate advanced electronic signal processing systems but

without artificial intelligence-based solutions. Low security stratosphere data centres do not incorporate advanced signal processing systems.

Influence on Avian Habitat: The deployment of data centres into the stratosphere should not endanger avian diversity at high altitudes. This is because some aves have been found to reside or migrate at high altitudes. For example, bar headed geese has been observed to fly at altitudes exceeding 8 km (Harrison, 2019) corresponding to the lower stratosphere region. The vulture and Whooper swan are noted to fly at an altitude of 11.278 km and 8.29 km, respectively. The influence on avian habitat is important due to biological conservation interests. Avian species that fly at high altitudes are mapped prior to deploying stratosphere data centres. This enables the development of a map for high altitude stratosphere avian habitats. An example of such a map is presented in (Parr et al, 2019). Stratosphere data centres are not located in stratospheric regions that support avian life. In addition, areas where stratospheric data centres disrupt bird's migration are not used as a long-term site for hosting stratosphere data centres. The information on avian life disruption is obtained from data on disturbance to the altitude and flight mechanism in stratosphere data centres.

The secondary criteria associated with the classification of stratosphere-based data centres are:

Number of Existing Aerial Applications: This is the number of applications with which stratosphere data centres share the aerial space. Examples of applications that utilize aerial vehicles in the stratosphere are: (1) Military (Harrison, 2019; Parr et al, 2019), (2) Wireless networks (Scott, 2017) and (3) Scientific Investigations (Massie, 2019).

Aviation Policy: The aviation policy describes the support by a country/sovereign region for the use of stratosphere aerial vehicles for data storage and access applications. The aviation policy also considers the compliance of the stratosphere data centre to data sovereignty policies.

Number of Service Providers: This is the number of service providers that own the servers aboard the deployed stratosphere data centre. The number of service providers influences the mobility requirements of a stratosphere data centre. A stratosphere data centre hosting computing payload belonging to a large number of service providers should be highly mobile to provide service to multiple subscribers.

Support for Open Computing: The information on the support for open computing indicates if the computing payload is non disaggregated, disaggregated or hybrid. A hybrid open computing payload supports non disaggregated and disaggregated servers. This factor has been considered to indicate the extent to which the stratosphere data centres embraces initiatives such as the open compute project. This is because disaggregated hardware and software play an important role in the open compute project (Dorn et al, 2018; ETSI, 2019).

Computing Payload Technology: The computing payload technology can be classified as non–neuromorphic, neuromorphic or hybrid computing payload (neuromorphic and non-neuromorphic).

Operational Stratosphere Temperature: The operational stratosphere temperature describes the stratospheric environmental temperature at the data centre location. A low and high temperature indicates that the stratosphere data centre benefits and does not benefit from stratospheric cooling, respectively. Benefitting from low temperature reduces cooling energy and enhances the PUE.

The primary and secondary criteria and the categories associated with the Tier classification of stratosphere data centres are shown in Figure 3 and Figure 4, respectively. In Figure 3, the primary criterion of the influence on avian habitat has two subcategories i.e. low environment threat (LETh) and high environment threat (HETh). LETh and HETh indicates that the stratosphere data centre does not and does pose any risks of avian extinction, respectively. The classification of stratosphere data centres hinges around aerial vehicle class and stratosphere temperature. The Tier classifications for stratosphere data centres are:

Tier 1a class A: The classification of stratosphere data centres also re-uses the terrestrial data centre's performance metrics of availability and redundancy. In addition, stratosphere data centre considers the choice of aerial vehicle and the threats posed to avian life. The availability and redundancy of a Tier 1 stratosphere data centre is similar to that of a Tier 1 terrestrial data centre. The general specification for the classification of a Tier 1 stratosphere data centre is given as Tier 1 *Alphabet* Class *Capital Alphabet*. A stratosphere data centre with Tier 1a Class A classification poses the least environment threat i.e. LETh (due to small letter *'a'*) to avian life and uses the category A plane (as specified in capital letter *'A'*). The additional stratosphere data centres with a Tier 1a classification are: Tier 1a class A, Tier 1a class B1, Tier 1a class B2, Tier 1a class C, Tier 1a class B2L, Tier 1a class B3 and Tier 1a class L (considering all sub–categories in class L). All the stratosphere-based data centres with the Tier 1a prefix have the same availability and redundancy, and a low environment threat for a given aerial vehicle class.

Tier 1b class A: The Tier 1b class A stratosphere data centre utilizes the class A plane with HETh to avian life. The availability and redundancy configurations are the same as that of the Tier 1 terrestrial data centre. Information on values of associated with primary and secondary criteria are specified as metadata. Additional categories in Tier 1b are Tier 1b class A, Tier 1b class B1, Tier 1a class B2, Tier 1b class C, Tier 1b class B2L, Tier 1b class B3 and Tier 1a class L (considering categories in class L).

The stratosphere data centre with availability and redundancy similar to Tier 2 terrestrial data centre (LETh) is given as Tier 2a class A, Tier 2a class B1, Tier 2a class B2, Tier 2a class C, Tier 2a class B2L, Tier 2a class B3 and Tier 2a class L (considering sub–categories in class L). In addition, the Tier 2b class A data centre (HEth) is similar to the Tier 2a class A data centre. In this case, the stratosphere data centre classifications are Tier 2b class A, Tier 2b class B1, Tier 2b class B2, Tier 2b class C, Tier 2b class B2L, Tier 2b class B3 and Tier 2b class L (considering categories in class L).

The proposed architecture uses a similar classification approach that recognizes the threat to avian life and class of aerial vehicles for the Tier 3a class A stratosphere-based data centre. In addition, the proposed Tier re-classification architecture for Tier 4 stratosphere data centres. The classification approach considers the Tier 4 classification (indicating the level of availability and

redundancy which is similar to terrestrial data centres), small alphabet (to indicate the level of threat to avian life) – with letter a being the least and indicating low environmental threats; and letter *c* being the last alphabet (in the classification consideration) and showing high environmental threats to avian life. The capital letter with possible values of A, B1, B2, C, B2L, B3 and L indicates the class of the aerial vehicle used to realize the stratosphere-based data centre.

A comparison of the different Tiers for stratosphere-based data centres is shown in Table II. In Table II, criteria whose parameters can have a wide range of values without loss of performance have their variables specified as variable.

Metric	**Tier 1a Class A**	**Tier 1a- Class B**	**Tier 1a Class C**	**Tier 1b Class A**	**Tier1b Class B**	**Tier 1b Class C**
Aerial Vehicle Class	A	B	C	A	B	C
Renewable	Yes (Solar)	Yes (Solar)	Yes (Solar)	Yes (Solar)	Yes (Solar)	Yes (Solar)
Threat to Aves	Mild	Mild	Mild	Moderate	Moderate	Moderate
Stratospheric Location	Variable	Variable	Variable	Variable	Variable	Variable
Number of Computing Platform Service Providers	Variable	Variable	Variable	Variable	Variable	Variable
Security and Threat Arising from Physical Exposure	Variable	Variable	Variable	Variable	Variable	Variable
Value of secondary criteria parameters	Variable	Variable	Variable	Variable	Variable	Variable

Table II: Criteria values and comparison for Tier 1 stratosphere-based data centre.

The proposed Tier classification architecture differs from the Tier classification of terrestrial data centres. This is because the Tier classification of terrestrial data centres essentially focuses on

data centre facility performance without necessarily considering the facility housing the data centre as seen in (OPNFV & OCP, 2018). This is because buildings do not pose significant threats to marine and avian diversity. This is because existing terrestrial data centre Tier classification does not consider factors such as security, use of renewable energy and networking ability. Newer tier classifications of terrestrial data centres consider the ability to support new networking standards and technologies such as open networking and computing described in (OPNFV & OCP, 2018; Khan, 2019).

In addition, the Tier rating of ocean and stratosphere data centres consider environmental friendliness (sustainability) as a criterion. Environmental friendliness is also recognized in the Tier 5 data centre classification which is presented in (Switch, 2020). However, these factors are considered in the novel Tier 5 terrestrial data centre classification. Table III presents a criterion-based comparison for the classification of the novel Tier 5 terrestrial data centre classification with that of ocean and stratosphere-based data centres.

Table III: Comparison of feature-based criteria for Tier 5 Terrestrial, Ocean and Stratosphere Data Centres.

Criteria	Tier 5 Terrestrial data centre	Ocean data centre	Stratosphere data centre
Availability	Yes	Yes	Yes
Redundancy Configuration	Yes	Yes	Yes
Vessel Class	No	Yes	No
Aerial Vehicle Class	No	No	Yes
Security	Yes	Yes	Yes
Missile threats	No	Yes	Yes
Marine Diversity	No	Yes	No
Avian Diversity	No	No	Yes
Renewable energy	Yes	Yes	Yes
Networking	Yes	Yes	Yes
Computing Payload Consideration	No	Yes	Yes

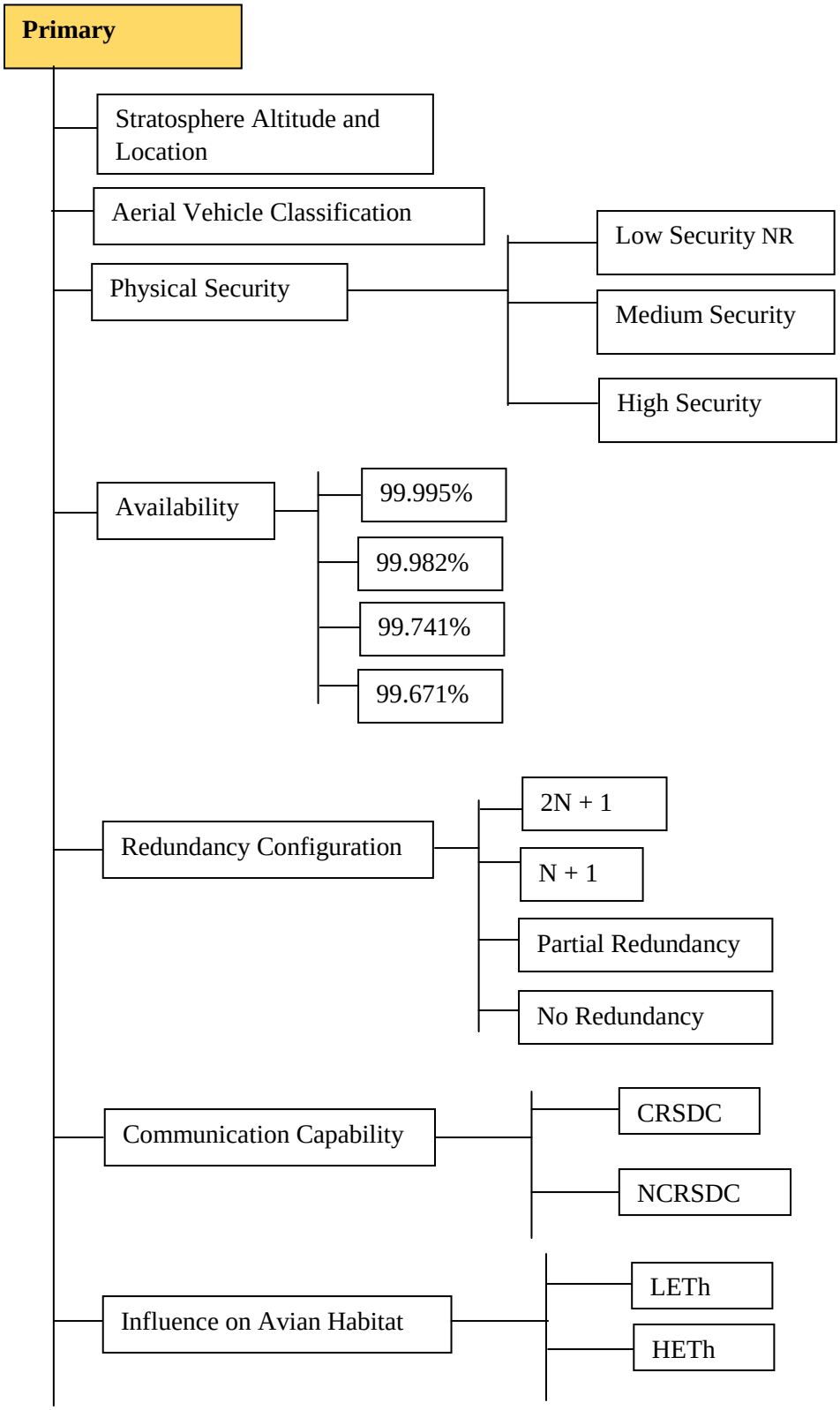

Figure 3: Primary Criteria for the Tier Classification of Stratosphere based data centres.

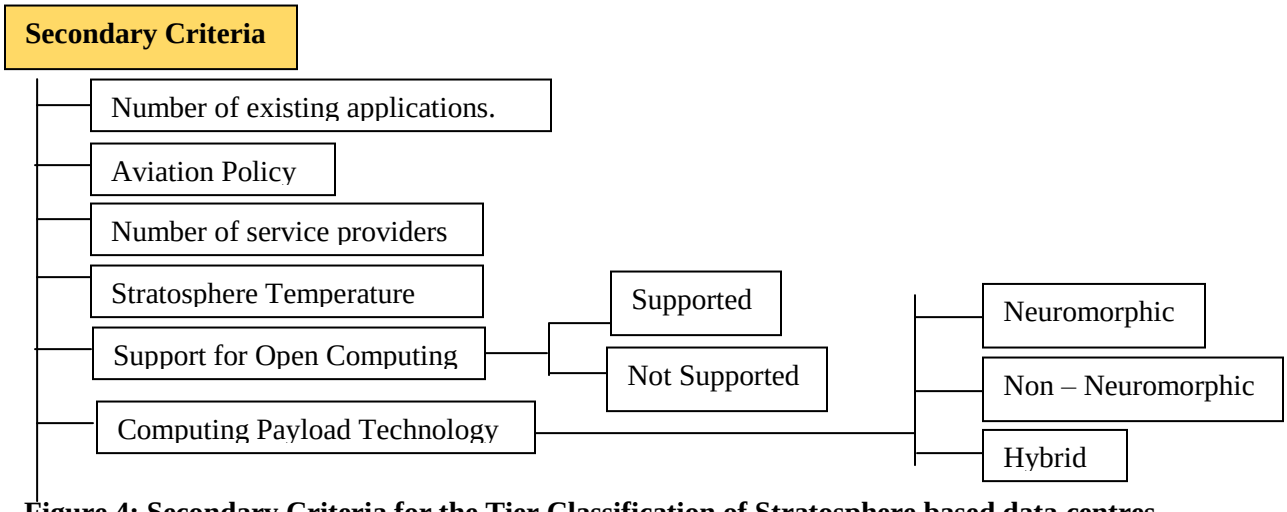

Figure 4: Secondary Criteria for the Tier Classification of Stratosphere based data centres.

Conclusion

This article proposes a novel Tier re-classification architecture for non–terrestrial data centres. The proposed re-classifications consider future non–terrestrial data centres located in the ocean and stratosphere. It classifies criteria to be used in the novel tier re-classification as either primary criteria or secondary criteria. The discussion in the paper identifies the primary criteria and secondary criteria for ocean-based data centres and stratosphere-based data centres. In addition, Tier classifications for data centres sited in the stratosphere and ocean have been presented and the roles of different criteria are also discussed. Furthermore, the proposed tier re-classification is compared with the existing Tier 5 terrestrial data centre and it is shown that the proposed classification architecture identifies the role of previously unconsidered factors. Additional work aims to further investigate the feasibility of non–terrestrial data centres with the proposed tier re-classification architecture.

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ORIGINAL RESEARCH ARTICLE

On the development of UJ-MaGT scientific calculator

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Abstract

Graphing is an important skill/knowledge required by almost every scientist, engineer and other professionals that require analysis of data to make sense of phenomena, relationships, etc. This important knowledge is learned right from secondary school through to advanced level of education and it is developed through the study of functions in mathematics and laboratory exercises in sciences, etc. At the secondary school level, learning to graph by hand is the most preferred and common practice in most countries in the African continent and other parts of the world. However, studies have shown that graphing by hand present numerous difficulties to science students due to its high "procedural load". To reduce the procedural load, an algorithm called UJ-MaGT was developed and tested for effectiveness. Excellent result in time management, simplification and pedagogical change in graph plotting process was achieved with the help of UJ-MaGT. Currently, the algorithm has been incorporated into a scientific calculator.

Key Words: *Graphing, Graphing Difficulties, Graphing Calculator, Science Education, and Graphing Pedagogy*

Introduction

The Problem

Graphing is a very important tool used in the analysis and interpretation of data by researchers and analysts in general; and scientists in particular (American Association of Physics Teachers, 2014; Woolnough, 2000). It helps in visualizing theories, algebraic functions, and phenomena. One cannot learn the complete syllabus of physics or mathematics in secondary school without learning graphing. It is made an integral part of laboratory activities in the curriculum of physics and mathematics courses/subjects in most, if not all, the countries of the world. Most curricula around the world introduce graphing to students right from JSS3 (Grade 9). Students start by

learning how to construct graphs and subsequently how to interpret and make sense of graphs. Graph construction using hands is usually emphasized for beginners.

The procedures of construction of graph by hand include drawing and labelling of axes correctly, choosing of suitable scales and scaling the axes correctly, plotting data points, drawing line of best fit, determination of slopes and intercept of the line of best fit, etc. Significant number of research have documented that some of these graph construction procedures present significant challenges to many students. Some of these challenges faced by students include the setting up of scales, plotting points at the correct locations, reading the coordinates of a point from the graph correctly, drawing best-fit line, interpreting of graphs etc. (Ryan, *et al*, 2016; Kola, 2013; Adolphus and Aderonmu, 2013; Roth &McGinn, 1996; and Wavering, 1989; Brasell & Rowe, 1993; Van Zee & McDermott, 1987; Bowen &Roth, 2005; Forster, 2004; Leinhardt, Zaslavsky & Stein, 1990; and Brasell, 1987). Ryan, *et al*. (2016) attribute some of these difficulties to high procedural load associated with graph construction by hand, of which scale setting constituted the highest difficulty. These challenges contribute to poor performances in examinations and loss of interest in learning of concepts involving graph construction (waeconline.org.ng, 2017; Jackson, Edwards, & Berger, 1993; Hattikudur, *et al*., 2012; Kali, 2005)

The solution

Technological solution available includes graphing calculators such as TI-84, TI-Nspire CX, Casio etc. These do not support graph construction by hand and many educators still have reservations on some of the technological advancements in these calculators. They are mostly programable and therefore can be useful tools for examination malpractice; and are very expensive beyond the affordances of students from many developing countries. Study by Brown, *et al*., (2007) revealed that some teachers view the use of graphing calculator as a means of getting to the answer without understanding the mathematical process. A question posted on stackexchange.com (2016) – '…why don't we discard the traditional pencil and paper method of graph plotting in high schools and for freshers at colleges since there are many electronic devices doing the graphing…?' - saw over 90 percent of the respondents write in favour of manual construction of graphs, arguing that it leads to better understanding of what graph is as opposed to the use of electronic devices. Others, however, opined that graph construction using paper and pencil supports active learning which enhances learner's understanding of concepts being studied (Davidwees.com, 2012; McDermott, *et al*., 2014; and Freeman, *et al*., 2004).

UJ-MaGT algorithm is developed with the sole aim of procedural load reduction and support of graph construction by hand thereby sustaining conceptual understanding, retention, and higher order achievements that can be realized from active learning (Ryan, *et al.*, 2016; Berg & Phillips, 1994; McDermott, *et al*., 2014; and Freeman, *et al*., 2004). It assists students in choosing a suitable scale for any given set of data and paper size. A suitable scale by West African Examination Council (WAEC) is a scale that does not comprise odd numbers other than 1 & 5 or multiples and submultiples of odd numbers other than 1 & 5 and can make the data points cover at least one-third (1/3) and, in some instances, one-half (1/2) of the space provided for graph construction (see supplementary material). To use the app, a student needs to understand some few things about one's data and paper. The students must be able to identify the largest and smallest values of his set of data, know the number of centimeters on his paper, know the number of decimal places of his data, understand the trend between his variable. It also helps in

converting data sets into its millimeter equivalence thereby making the construction process much easier and within the WAEC accuracy limit which is plus or minus half of the millimeter box. This is easier because instead of locating a coordinate by tracing its value on the axis, the student just counts the number of millimeters equivalent to the desired number. Similarly, the student can use the algorithm to convert number of millimeter boxes into the corresponding value. This helps students to read the coordinates of right-angle triangle accurately when determining slope and reading intercepts. (See youtube video; https://youtu.be/7s7b2xNoaCM)

Purpose of the Paper

The aim of this paper is to present the various stages of the development of UJ-MaGT scientific calculator for the purpose of provoking interest and guiding future innovators of educational technologies.

Developmental Stages

Formulation

Mathematical equations (1) (2) and (3) (Mafuyai, *et al*., 2013a; Mafuyai, *et al*., 2013b) were formulated. These were formulated on the bases of number theory and guided by the rubrics of both West African Examination and National Examination Councils' (WAEC & NECO) physics practical marking scheme. This was necessary because both WAEC and NECO standards are the minimum most general standards of graphing accuracy required of the students of the member countries of WAEC.

$$K = 2^{m-x} \times 5^{\varepsilon-x} \qquad (1)$$

$$\lambda \approx \frac{NK}{Z-\mu} \qquad (2)$$

$$where\ \varepsilon\ is\ determine\ from\ {}^{2Z10^{x}}/_{N} \gg /5 = P_{\varepsilon} \quad for\ which\ P_{\varepsilon} < 5$$

$$\mu = Kv \qquad (3)$$

$$where\ v\ is\ determine\ from\ L = Kv + Kw$$

Validation: the work was presented to experts at the 35th Annual National Conference of the Nigerian Institute of Physics for criticism and thereafter, published in the journal of Nigerian Association of Mathematical Physics as cited above. A book chapter was written; detailing how the formulas can be used in graph construction and published in the textbook used for year one physics practical courses in the University of Jos (Ike, *et al*., 2015).

Algorithm and Executable File for Desktop computers

The algorithm/flowchart[1] based on equations (1), (2), (3) and other auxiliary equations was developed. This was converted into a computer programme and executable file using C# programming language by one of the co-authors in this paper. (Figure 1).

Validation: to validate the programme, approval was sort and granted by the vice chancellor of the University of Jos to install the programme on the University's library computers for students who needed to make use of it in the process of reporting their laboratory experiment. Feedback

[1] **See flowchart at the University of Jos repository**

from the library management[2] revealed that there was a massive increase in the use of library computers by students during the academic session. Opinions and suggestions from the students[3]at the end of the session were sort through questionnaires on areas requiring improvement. The algorithm functionality was improved as a result of some of the suggestions made by the students.

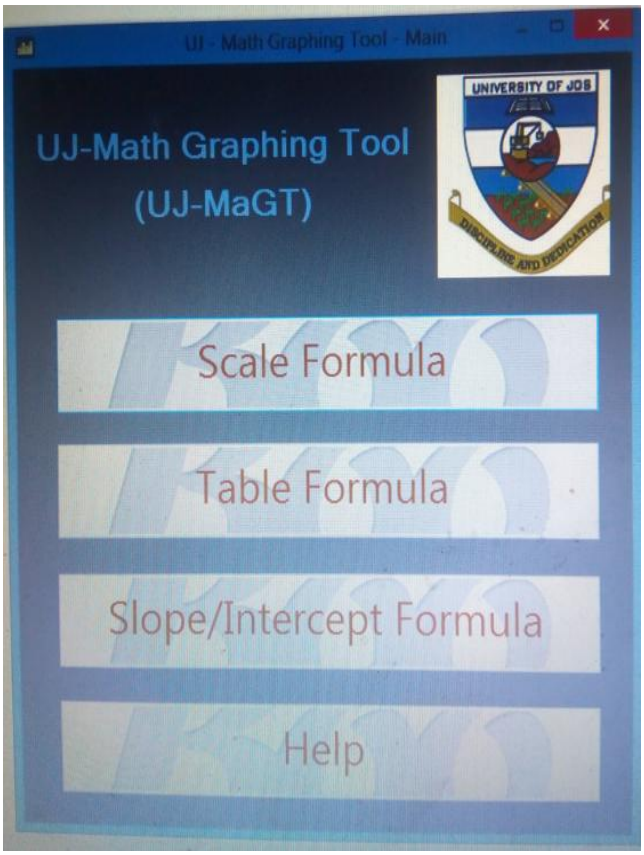

Figure1. Interface of the UJ-Math Graphing Tool (UJ-MaGT)

Mobile App

The algorithm was developed into a mobile app (Figure 2) to make it more portable and increase both affordability and availability. Three platforms were made; Android, iOS, and Blackberry (see Android version: https://play.google.com/store/apps/details?id=com.bitrient.magt). The mobile app enabled the validation and assessment of pedagogical impact of the algorithm on graph construction.

[2] **See appendix for the report from the library.**
[3] **See the compiled comments of the students in the University of Jos repository**

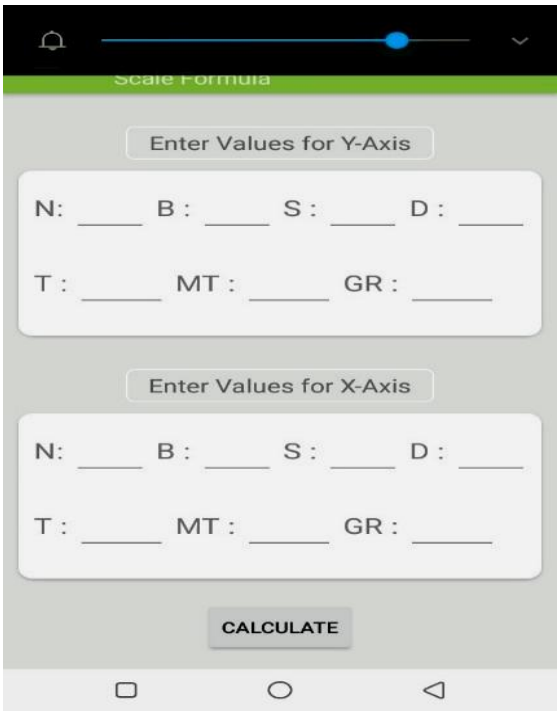

Figure 2: The Interface of the Scale Formula

Validation: to validate, a comparative study sponsored by Tertiary Education Trust Fund (TETFund) institutional-based research (IBR) grant was carried out. The study assessed the impact of the algorithm in improving performance as well as attitudinal and motivational impact (Mafuyai, *et al*., 2018). To assess performance improvement, the work compared two procedures of load reduction methods of teaching in graph construction by hand; the use of UJ-MaGT software and the use of specific guidance (Ryan, *et al*., 2016). First-year undergraduate students who registered for physics practical course consented and enrolled for the study. The group that was taught some specific instructions on graph construction is called "Specifics Instruction Group" (SIG) and the group that was taught how to use UJ-MaGT app (computer software) in graph construction is called "Computer Software Group" (CSG). A pre-treatments test was administered at the beginning of the semester while a post-treatment test at the end of the semester. The scripts were graded in accordance with the West African Examination Council's Physics practical marking rubrics. The result shows that the students' level of competence at the beginning of the semester was fairly similar as revealed by the mean values of their pre-test scores of 4.18 and 4.24 with average completion times of 14.23 minutes and 14.22 minutes for SIG and SCG respectively. The mean values of the SIG's post-test score and time of completion were 4.83 and 13.51 minutes which were not statistically significant ($P>0.01$) compared to the mean values of pre-test scores of both groups. The mean values of the CSG's post-test scores and time of completion were 11.39 and 8.52 minutes which were statistically significant ($P<0.01$) compared to the mean values of pre-test of both groups and post-test of the SIG. Large effect sizes (Coe, 2002) of up to 3 and 1.5 were achieved using UJ-MaGT as against 0.22 and 0.14 achieved by use of specific instruction in graph construction.

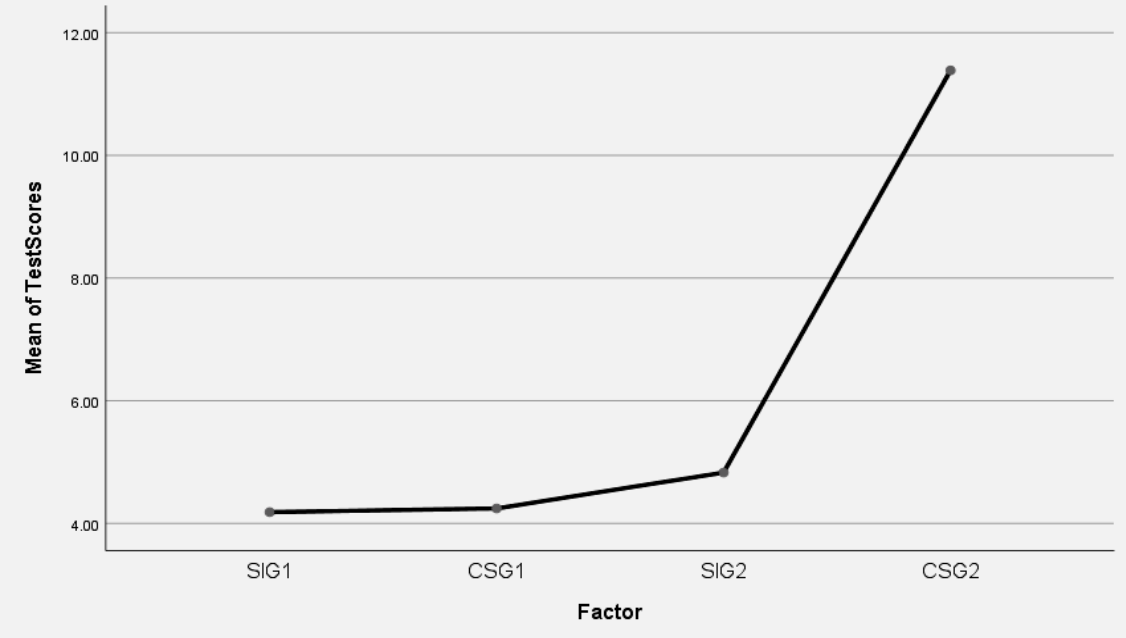

Figure 3[4]: Comparison of Mean Scores for each Group's Test Scores.

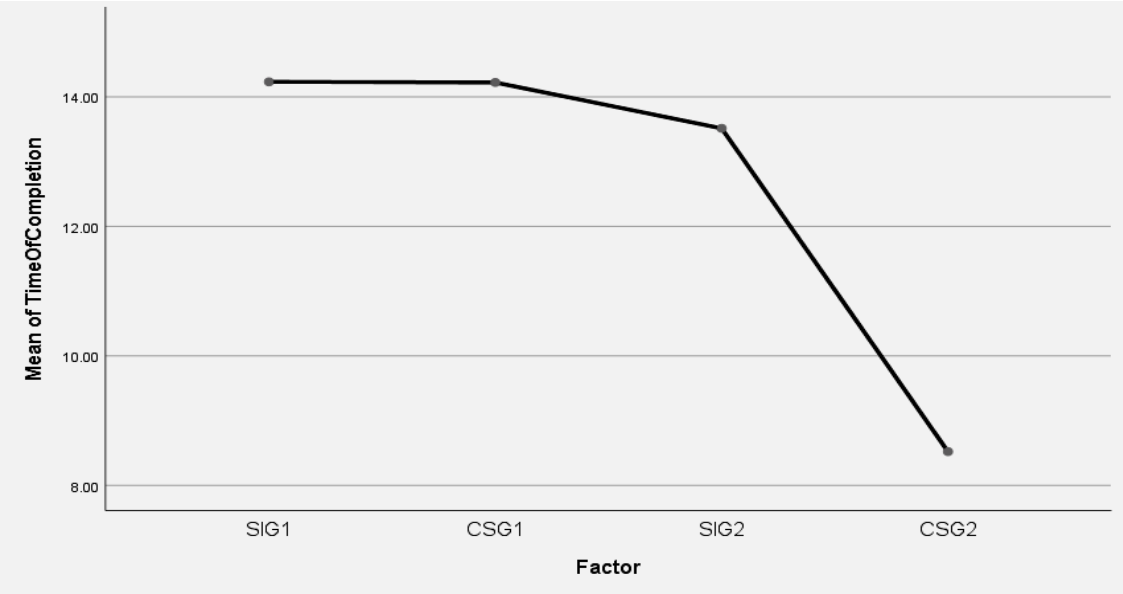

Figure 4[5]: Means Plot for the Time Taken to Complete Graph Construction

[4]The abilities of both groups in construction of graph at the beginning of the semester were fairly similar (SIG1 and CSG1). However, at the end of the semester, both groups improved in their abilities which lead to increase in mean scores. But the group that used UJ-MaGT (CSG) had the highest mean score (CSG2)

[5] The groups' time taken to construction a graph at the beginning of the semester were fairly similar (SIG1 and CSG1). However, at the end of the semester, both groups improved in their time management which led to decrease in time taken to construct a graph. But the group that used UJ-MaGT (CSG) had the lowest time (CSG2)

Furthermore, students' attitude and willingness to graphing was found using a questionnaire at the end of the semester following the use of UJ-MaGT. Result shows (Figure 5) improved attitude and willingness to engage in topics involving graph construction. In expressing their willingness to enroll for a graph plotting course, 88% said they will enroll if app is available while 12% said they will not enroll if app is available. And 29% said they will enroll if app is not available while 71% said they will not enroll if app is not available.

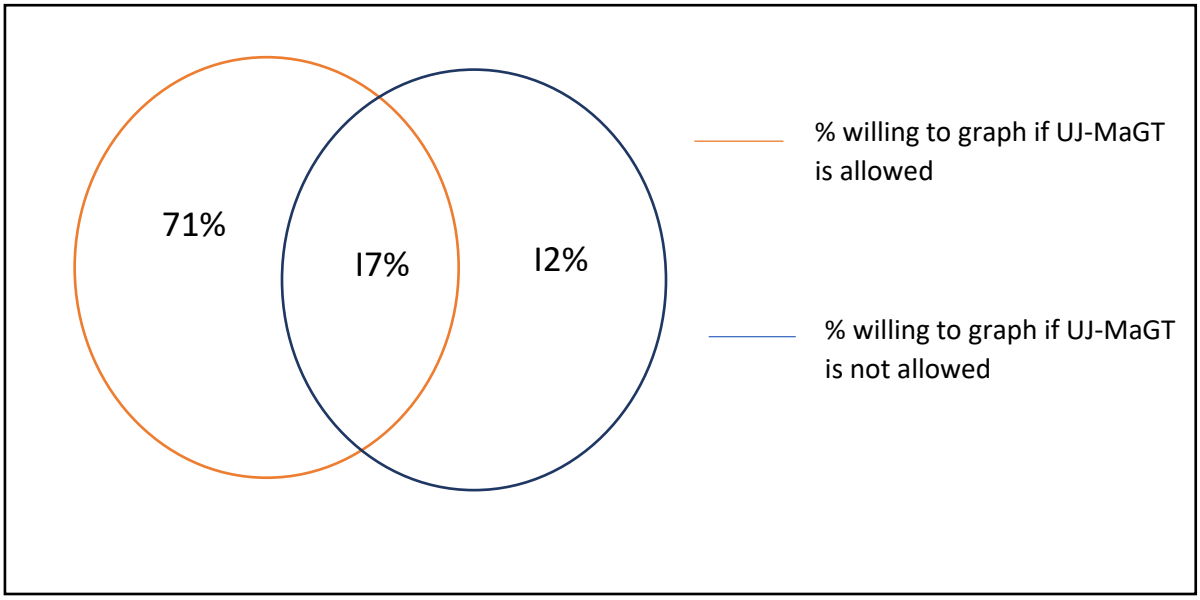

Figure 5: Venn diagram of Participants' Willingness and Attitude to Graphing Using UJ-MaGT

The motivational impact was assessed using modified educational motivation scale. Extrinsic motivation external regulation, extrinsic motivation identified regulation and intrinsic motivation toward accomplishment were the highest aroused type of motivation among the students (Mafuyai, *et al*., 2018).

Protection of Intellectual Property; the algorithm has been protected by the Nigerian copy right law through registration with the NCC[6].

UJ-MaGT Scientific Calculator

The algorithm has been incorporated into a scientific calculator to make it fit for use in secondary schools. The mobile app version of the calculator has been developed (Figure 6) and hardware is under construction through the support of Federal Ministry of Science and Technology.

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[6] See appendix for the copy right certificate

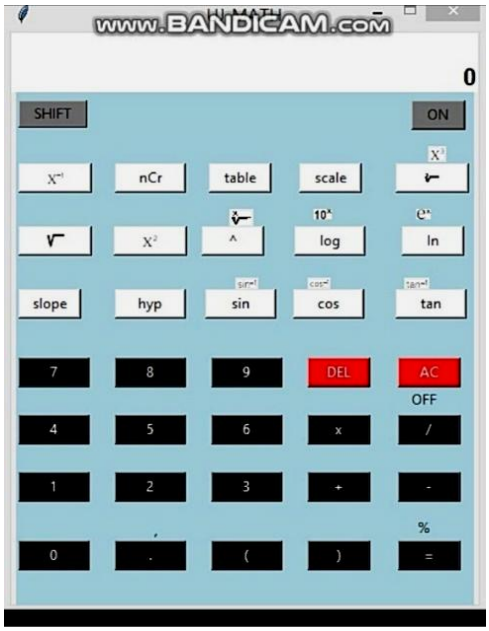

Figure 6: Interface of the Mobile App Version of the UJ-MaGT Scientific Calculator.

Validation: to validate the scientific calculator, a TETFund IBR grant has been secured to undertake a validation study among secondary school students.

Protection of Intellectual property; National office for Technology Acquisition and Promotion (NOTAP) has been consulted and processes are on for filing for patent.

Discussion

Roth and McGinn (1996) opined that teachers should not be mere implementors of classroom technologies but be involved in the development of the technology. This is necessary because the technology could be designed to optimize pedagogical gains and minimize chances for misused. For example, graphing calculators are programable and therefore, a potential tool for malpractice. UJ-MaGT Scientific Calculator on the other hand was designed with no such possibility. Furthermore, in the context of Nigerian and African quest to develop a science and technology-based economy, development of UJ-MaGT Scientific Calculator offers hope to young African Scientists/and entrepreneurs. This can serve as a source of inspiration and motivation for them to innovate and create solutions to problems using Science, Technology, Engineering, and Mathematics (STEM) knowledge they have acquired.

Conclusion

This work has presented and made clear the processes leading to the development of UJ-MaGT Scientific Calculator which could be adopted easily by any other innovators of educational technology. The most important steps include identification of a problem, available solutions and their limitations, creating a new solution, validation of the new solution, and protection of intellectual proper.

Acknowledgement
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Appendix

Nigerian Copyright Commission

Copyright Registration

Registration No. AW0097

The Nigerian Copyright Commission has been notified of a work with the following particulars:

Author of Work: MAFUYAI MABUR YAKS

Title of Work: UJ-MATH GRAPHING TOOL

Type of Work: ARTISTIC WORK

Year of Creation: 2016

Owner of Copyright: MAFUYAI MABUR YAKS

Issued 18 Day of July 2017

MICHAEL AKPAN
For: Director General

NIGERIAN COPYRIGHT COMMISSION

UNIVERSITY OF JOS
THE UNIVERSITY LIBRARY

Vice Chancellor
Professor Hayward B. Mafuyai
University Librarian
Stephen A. Akintunde
(Dip Lib; B.Sc (Hons), PGC (IM), M.Sc, PhD)

P.M.B. 2084,Jos,Nigeria
073-453734
Fax: 073-611928
E-mail:librarian@unijos.edu.ng

August 27, 2015

REF: /UJL/33G

To: Mafuyai Mabur Yaks ✓

RE: PERMISSIONTO INSTALL MAFUYAI GRAPHIC TOOL (MaGT) IN THE LIBRARY COMUTERS OF THE UNIVERSITY WITHIN MAIN CAMPUS.

With reference to your memo on the above subject dated **20th May, 2015**. I hereby submit my report on the use of the software in the library as requested by the course lecturer.

The MaGT graph was installed on a total of 61 computer systems in the Bauchi Road Campus Library. Take-off was initially slow with low patronage as most of the students did not know what to do and how to use the software.
Eventually, the use of the MaGT software stabilized, and the Systems Unit of the library had to install the software on more computer systems due to popular demand.

Users of the graph come in groups as assigned by the course lecturer. Their graphs are plotted and students leave for another group to take over.

Akpokodje, Vera N. (Mrs.)
Systems Librarian

CC: University Librarian
HOD, Physic Department

ORIGINAL RESEARCH ARTICLE

Optimization of polyphenols extraction from sweet potato peel: single factor versus chemometrics approach

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ABSTRACT
The study was aimed at optimizing the extraction conditions of phenolic compounds from an orange-fleshed cultivar of sweet potato peel using a single factor vis-a-vis chemometric experimental approach. The effectiveness of extracting antioxidants compounds using an aqueous medium (water) was compared to organic solvents. Water-to-peel, time of extraction and extraction temperature was established to have significant effects on the total antioxidant and scavenging activities. Optimal extraction variables of 60 ºC for 20 minutes, at a solvent-to-peel powder ratio of 100:7.5 mL/g was derived for the process. Approximately 157.0 g of peel powder was obtained from a kilogram of the dried potato peel using a simple but scalable method. The polyphenol isolate contained 31.39 % polyphenols and compared significant well with vitamin C and butylated hydroxytoluene (BHT) in terms of its antioxidant activities. In summary, this study used an aqueous medium as its extracting solvent, which offers a great prospect for future use in the extraction and purification of bioactive compounds from plant materials.

Keywords: *Antioxidants; Bioactive component; Ipomoea batata speel; Chemometrics; Extraction; Polyphenols*

INTRODUCTION
Sweet potato is a sweet-tasting food crop with rich nutritional properties (Mohanraj and Sivasankar, 2014). It is grouped under the family Convolvulaceae and its tuber has high starch content (Woolfe, 1992; Mohanraj and Sivasankar, 2014). The edible tuberous root is long, tapered and smooth with a maturity period of between 90 to 120 days.

Antioxidants from natural source had continued to attract growing attention because of their potential health benefits (Arun *et al.*, 2015; Wijngaard *et al.*, 2009). They have been known to prevent lipid oxidation, deterioration of food and use in food quality preservation (Alamed *et al.*, 2009). They are also known to be a unique set of additives which can preserve and extends food shelf life (Shahidi and Ambigaipalan, 2015). While natural antioxidants are extracted from mostly plant material, synthetic antioxidants are made from common additives known to retard oxidative reactions. Synthetic antioxidants had been reported to have a harmful effect on human

health (Mohdaly *et al.,* 2010) which may lead to carcinogenic onset in humans. Therefore, exploration of natural antioxidants used in food application has gained interest in recent times.

Several studies had reported the possibility of extracting healthy compounds such as fibre, bioactive compounds, natural antioxidants and natural food additives from plant by-products (Al-Weshahy & Venket, 2009; Kadiri *et al.,* 2017). Commuting plant wastes and post-harvest losses to bioactive production would be an appreciable benefit to food manufacturers (Moure *et al.,* 2001; Wijngaard *et al,* 2009; Kadiri *et al.,* 2017) since it will save time and reduces the economic cost of disposal. Amado *et al.* (2014) in a prior study optimized antioxidants compounds extraction from the peel of *Solanum tuberosum* using the response surface methodology (RSM). Though the optimum yield of antioxidant was achieved at 89.9 ºC, 34 minutes and 71.2% and 38.6%, this study was however limited with the use of ethanol as extracting solvent which may not be approved by most food and health agencies of several countries. Recent advances in methodologies in potato peel waste management studies have been successful, including procedures such as microwave-assisted extraction (Singh *et al.* 2011; Wu *et al.,* 2012) and pressurised liquid extraction (Singh & Saldana, 2011; Wijngard *et al.,* 2009). The high cost of equipment and operation of this process have however limited their application in the agro-food industry (Amado *et al.,* 2014).

The peel of potato has been reported to have a higher concentration of phenolic compounds and has been recommended for use in the production of bioactive compounds like polyphenols (Amado *et al.,* 2014; Kanatt *et al.,* 2005). Previous studies had focused on the use of organic solvent for the extraction of bioactive compounds from plant materials with less emphasis on water extraction. There is the premonition that water medium has lesser extracting capability for bioactive compound compared to organic solvent. But in such instances, water might be made an effective medium for extraction in a situation where processing variables such as extraction time and temperature are optimized. Vuong *et al.* (2013) was able to show that water as an extraction medium gave higher polyphenol yield from the leaves of *Carica papaya* compared to other organic solvents like acetone, methanol and ethanol. Besides its safety and acceptability, water is free and universally available. This study hypothesizes that water as a medium for the extraction of antioxidants from sweet potato peel will give higher antioxidant yield and activities when processing variables and conditions are optimized in a single factorial experimental design.

Materials and methods

Materials

Sweet potato tubers (*Ipomoea batatas*) were obtained from the Obafemi Awolowo University, Ile Ife, Nigeria. The peels were mechanically peeled from the tuber, dried in a locally fabricated cabinet dryer at 55 ºC for 8 h. The dried potato peels were ground using an attrition mill, sieved and stored in a ziplock bag. This was labelled PP. Chemicals used for the analysis were purchased from the Sigma Aldrich Company (UK).

Experimental designs on polyphenol yield determination

The effect of extraction temperature on polyphenol yield was determined at the temperature range of 50 – 90 ºC. Exactly 2 g of PP was extracted in 200 mL aqueous medium in a shaking thermostatic water bath for 20 minutes. The optimal temperature (T; 60 ºC; results are displayed in section 3.1) which was the temperature where the highest yield of antioxidant activity was

recorded and thereafter use in the determination of the optimal extraction time. Exactly 2g of PP was extracted with 200 mL aqueous medium at a time interval of 5 to 65 minutes and the time of extraction which gives the highest antioxidant yield was noted as the optimal time (t: 20 minutes; results are displayed in section 3.2). The optimal time and temperature derived were then applied in the determination of the water-to-peel ration which gave the optimum yield of antioxidants. Water-to-peel ration (E) of 100:1, 100:2, 100:2.5, 100:5, 100:7.5 and 100:10 aqueous extraction at 60 ºC and 20 minutes was carried out and the optimum solid to water ratio determined.

Aqueous extraction efficiency was compared with organic solvents (acetone, isobutyl alcohol, ethanol, methanol and diethyl ether). Ground potato peel of 7.5 g was extracted in 100 mL of water (see section 3.3) at 60 ºC for 20 minutes or in 100 mL of organic solvent at room temperature (RT) for 4 hrs. The optimum processing variables (T: 60 ºC; t: 20 minutes; E: 7.5 g: 100 mL) described previously was adopted in the preparation of polyphenol isolate (PI) from PP. The process describing this production is as shown in Figure 1.

Determination of the peel composition

The procedure described by Vuong *et al.* (2013) was adopted for the preparation of the PP and PI prior to their analysis for their peel composition. The method reported by Skergetˇ *et al.* (2015), Zhishen *et al.* (1999), Li *et al.* (2006) and Hiai *et al.* (1976) were adopted for the determination of total polyphenol (Tp), Total flavonoid (Fv),Proanthocyanidins (Pro) and Saponins (Sp), respectively. Free-radical scavenging activities (DPPH) and total antioxidants activities (TAC) were determined by following the methods described by Thaipong *et al.* (2006) and Prieto *et al.* (1999).

RESULT AND DISCUSSION

Extraction temperature effect on antioxidant yield and activities

Figure 2A shows that increasing temperature from 50 ºC to 60 ºC results in increasing polyphenol yield. The yield nonetheless diminishes when the extracting temperature was extended beyond 60 ºC, an observation which might be connected to the decomposition of polyphenol compounds induced by thermal heat at a higher temperature. Earlier studies have established a direct relationship between temperature and extracted bioactive compounds (Vuong *et al.*, 2013; Alu"datt *et al.*, 2011; Ballard *et al.*, 2009; Pinelo *et al.*, 2005). While Gertenbach (2001) reported that greater solvent temperature normally leads to increase mass transfer rates during the process of extraction, Vuong *et al.* (2010) reported that temperature at elevated instances triggers opposing processes such as epimerization and decomposition of polyphenols. In view of this, there is the need for strict monitoring of the temperature of extraction of bioactive compounds so as to minimize polyphenols loss induced by thermal decomposition.

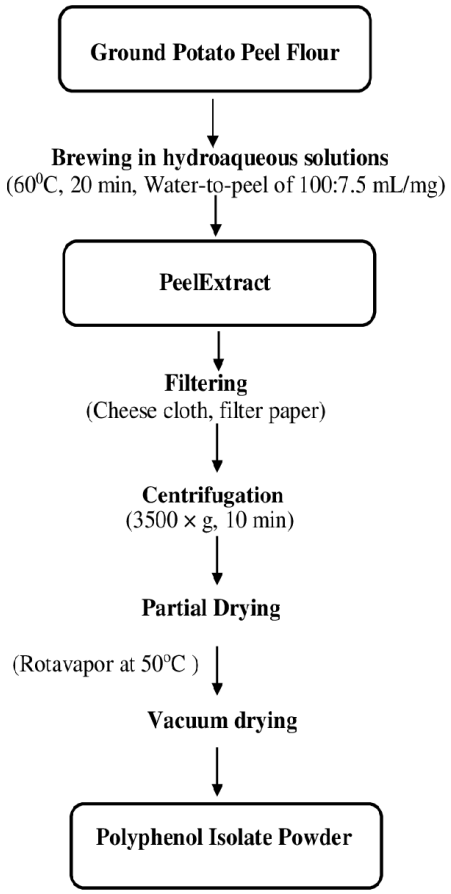

Figure 1 – Diagram of the method used to prepare polyphenol isolate of sweet potato peel leaf powder.

Influence of extraction time on antioxidant yield and activities

The influence of varying extraction time on PP polyphenol extractability, stability and yield are shown in Figure 2B. Polyphenol yields were observed to increase with extraction time within the interval of 5 – 20 minutes, beyond which the phenolic compound concentration was observed to decrease. The same trend was observed with antioxidant activities where the optimum time was also observed to be set at 20 minutes. Optimal yield of phenolic and flavonoid compounds was attained at an extraction time of 34 minutes in a prior study (Amado *et al.,* 2014), which is significantly higher than our reported optimum time (20 minutes). This shows an improvement of 70 % efficiency in the extraction time. Extraction time has been reported to significantly affect phenolic compounds extractability in peanut skins (Nepote *et al.,* 2005), while improvement in phenolic compounds and antioxidant capacities with increasing time have been reported in olive seeds (Alu'datt *et al.*, 2012).

Influence of water-to-peel ratio on antioxidant yield and activities.

The extent of water-to-peel ratio on phenolic compounds extractability, scavenging activities and total antioxidant capacities are presented in Figure 2C. An increasing trend in TP, Fv, DPPH and TAC was observed with an increasing water-to-peel ratio (per 100 mL solvent). Amado *et al.*

(2014) reported similar findings on the optimization of antioxidant extraction from *Solanum tuberosum* potato peel. Gertenbach (2001) suggested that as water to peel ratios increases, higher concentration gradients are created between phenolics trapped inside the peel cellular organelles and those at the surface regions, resulting in accelerated extraction kinetics.

Notwithstanding, significant energy input is required to optimize polyphenol yields using high dilution aqueous extracting medium and techniques. The reason has been that water has significantly higher heat capacity (J/g/K) which is about twice that of generally utilized organic solvents (H_2O = 4.18, acetone = 2.17, methanol = 2.53, ethanol = 2.44) (Cracolice and Peters, 2009; Vuong *et al.* 2013). Consequently, with consideration to the precise reason and volume of material to be prepared, a suitable water-to-peel ratio can be chosen. Based on the outcomes of this study, a ratio of 100:7.5 mL/g was appropriate for the determination of *Ipomoea batatas* peel composition, while a ratio of 100:5 is suggested for preparing its peel powder for further use. Extraction of polyphenol from the *Ipomoea* peel at this ratio can save 5.0 times of water volume and requires significantly lower energy to heat the water as well as to concentrate the extract by the removal of moisture to obtain its flour. Vuong *et al.* (2013) stated that lower solvent ratio to solid extraction is not appropriate as this will lead to considerable loss of phenolic compounds as well as increased difficulty in the separation of extracted solid from its extracts.

Validation of predicted optimum extraction condition variables

To validate the various optimal predicted points in section 3.1, 3.2 and 3.3 for temperature, time and solid-to-solvent ratio, a chemometric approach was adopted using the principal component (PC), hierarchical cluster (HC) and k means clustering analysis. Figure 2a-c shows the dendrogram following a hierarchical cluster analysis in which two well-defined clusters are visible. Samples were cluster in two groups in respect to their degree of similarity or nearest of characteristics. The first cluster in Figure 2a consists of just polyphenol compounds which is a result of the significant effects of temperature variability on bioactive extraction. The cluster formed a link with a second cluster consisting of TAC and SA, respectively. These observations were further validated in the K means profile plot shown in Figure 3A. The TAC and SA activity may be well close together due to similarity in their antioxidant activities while PC in cluster one has a direct influence on their activities.

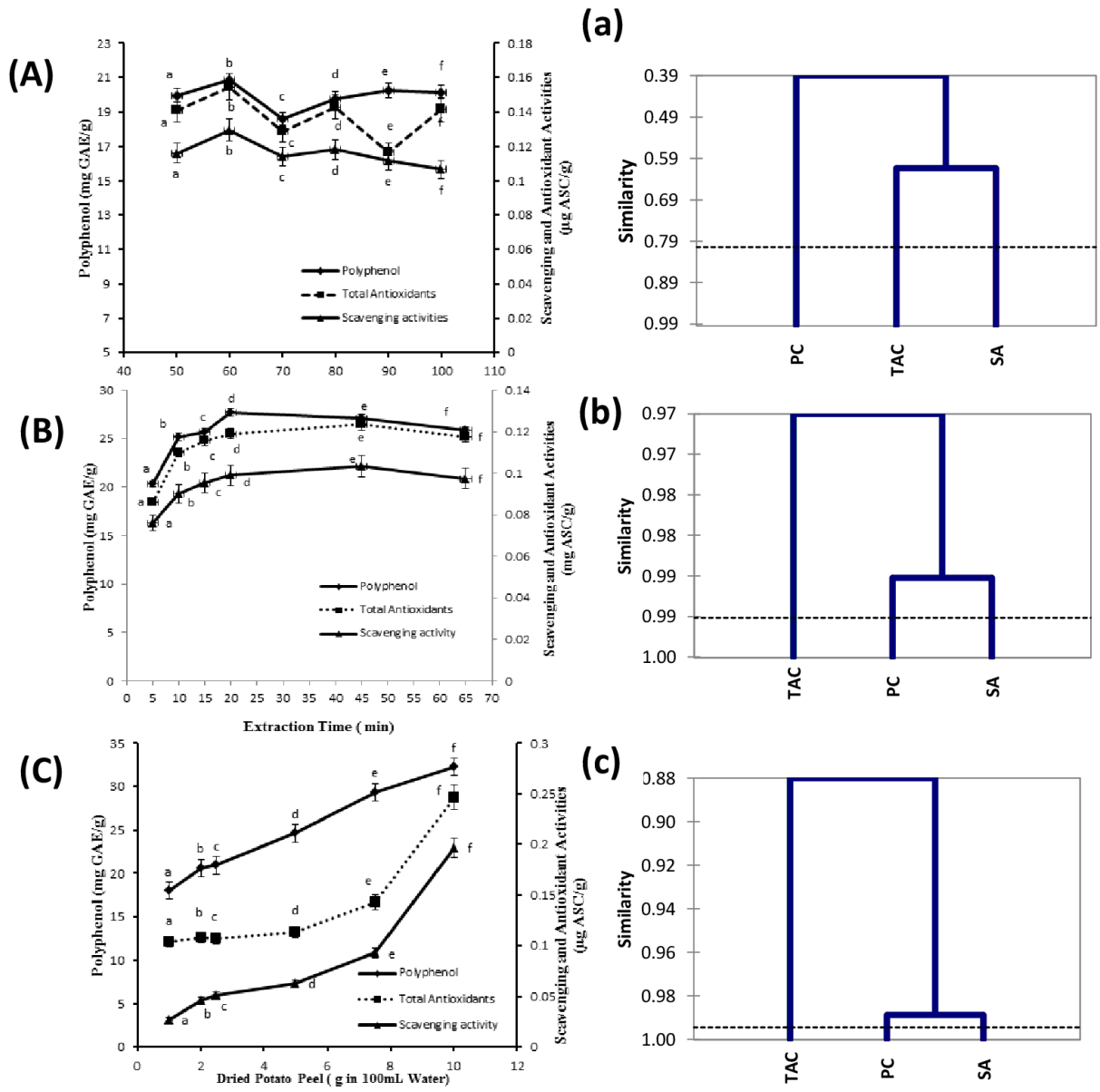

Fig. 2 – Effect of temperature (A); extraction time (B); and water-to-peel ratio (C) on the extraction yield of sweet potato peel polyphenols (left axis) and on the free radical scavenging and total antioxidant activities (right axis). The values are mean ± standard deviations for extractions values and those not sharing a letter are significantly different at $P < 0.05$; Hierarchical cluster analysis (a) Temperature (b) Time (C) Solid to solvent ration of TAC, PC and SA.

In Figure 2b, the HC partitioned the samples into two clusters based on the similarity of characteristics. TAC formed the first cluster while PC and SA formed the second cluster. In terms of closeness, PC and SA were more similar due to their cluster in group 2. The TAC in cluster one directly linking to cluster two implies that these two have a direct effect on the outcome of scavenging abilities and polyphenol compounds of the antioxidant extracts. The

same trend was observed in Figure 3C, but with lesser similarity with TAC and PC when compared with TAC and PC recorded in Figure 3b. These observations were further validated in the K means profile plot shown in Fig. 3 A-C, where PC, TAC and SA showed similar interaction reported previous in Figure 2a-c.

Principal component analysis was conducted in other to understand which factors (time, temperature and solid-to-solvent ratio) had the most profound effect on the active variables (TAC, SA and PC) and to what degrees. Data on the polyphenol and antioxidant properties of the peel extracts were subjected to PCA and shown in Figure 3 a-c. The multivariate treatment of the data acquired for the effect of temperatures on the total antioxidant capacities (TAC), scavenging activities (SC) and polyphenol compounds (PC) reduces the factors to two principal components (PCs), which described 87.46 % of the overall data variability. The first axis accounted for 65.4% and the second axis for 22.42 %. The factors loading are also displayed on the PCs loading in Figure 3A-C. As regard Fig.3A, polyphenol compounds were positively correlated to PC1 axis while TAC, SC were negatively correlated to the PC2 axis which has the loading for temperatures at 50 ºC, 80 ºC and 60 ºC with 60 ºC been the most significant correlated variable. Antioxidant extracted at 60 ºC was rated high in SA and TAC while PC was not associated with any temperature. Biplot after Varimax rotation (plot not shown) clearly shows a high clustering of TAC, SA and PC with 60 ºC which implies that a strong correlation exists between these terms. This temperature (60 ºC) was previously reported in section 3.1 of this study as the optimum extraction temperature.

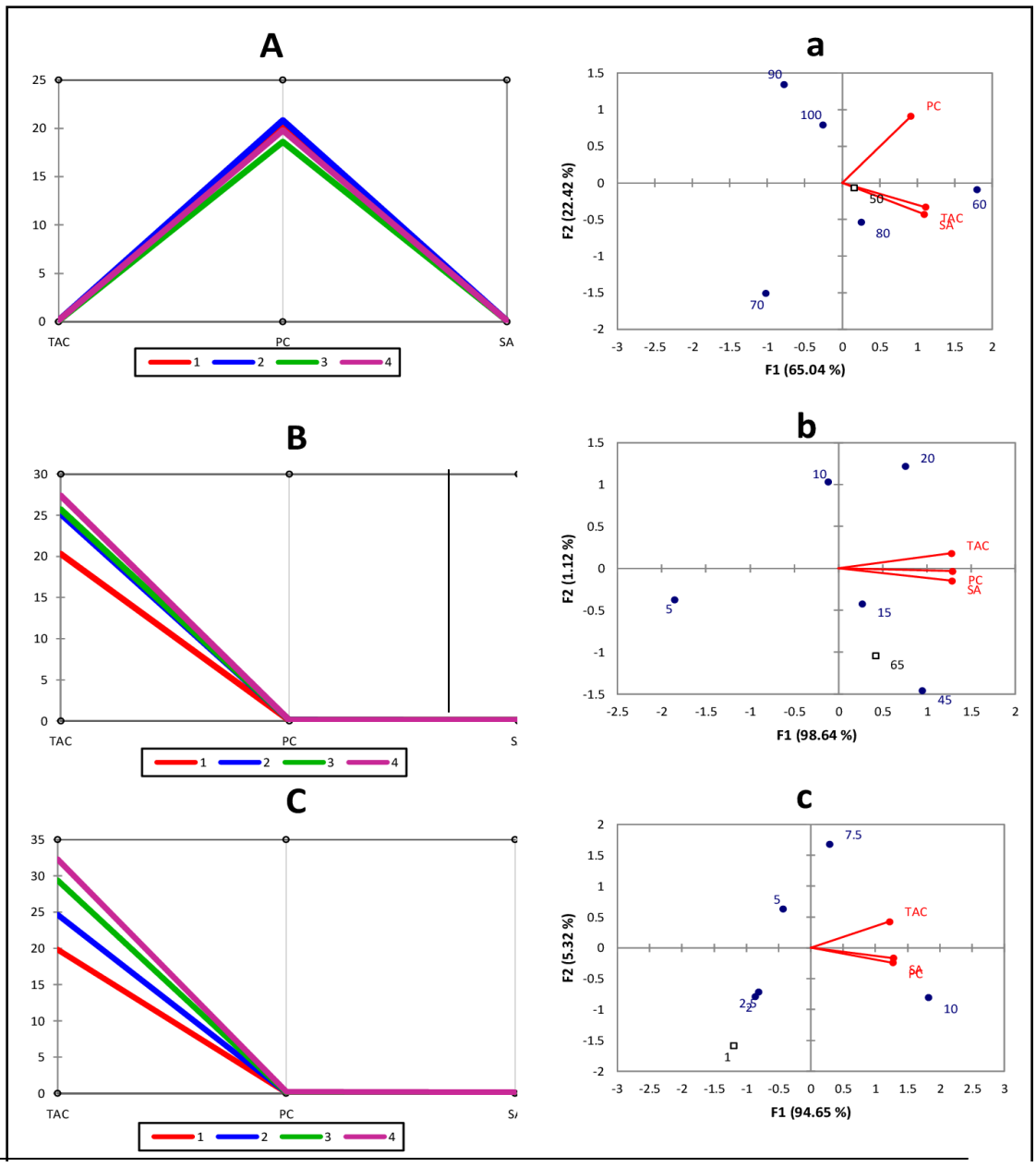

Fig. 3: Profile Plot of K Means Clustering of the Effect of (A) Temperature (B) Time (C) Solid to solvent ratio on Total antioxidant activity (TAC), Polyphenol (PC) and Scavenging activity (SA). Sample cluster: (a); 1: 50, 90 & 100 ^{0}C; 2: 60 ^{0}C: 3; 70 ^{0}C; 4: 80 ^{0}C / (b); 1: 5 Minutes; 2: 10 minutes; 3: 15 & 65 minutes; 4: 20 minutes and 45 minutes/ (c): 1:: 1,2,& 2.5%; 2: 5%: 3: 7.5%; 4: 10 %.

The principal component of the multivariate treatment of data accounting for the effect of extraction time and solid to the solvent ratio on total antioxidants capacity, scavenging activities and polyphenol content is shown in Figure 3b and 3c. The multivariate treatment allowed for the reduction of the factors to two PCA which described 99.76 % and 100.27% of the total data (Figure 3b -c). In Figure 3b, F1 accounted for 98.64% while F2 accounted for 1.12%. In Figure

3c, the first and second axis (F1 & F2) accounted for 94.65% and 5.32% of the total variance in the data set. The PC1 was entirely correlated with the variables in both instances than PC2. In figure 3b and 3c, TAC, SA and PC was an active variable factor which was located in the PC1 axis. For the case of Figure 3b, it was observed that TAC, PC and SA were located a distance from extraction time of 20, 15 and 65 minutes. Considering the time of extraction, 65 minutes was not an active factor, while 20 minutes was located on the positive axis of the PC1. In Figure 3c, the active fixed factor (7.5, w: v) was located on the positive axis of the PC1 and was close to the active variable factors of TAC. The PC and SA form a cluster at the negative axis of the PC1 and were in close proximity to the TAC and active variables of 15 and 20 minutes. This suggest that these antioxidant characteristics were positively correlated to 20 minutes of extraction on the positive axis of the PC1, a confirmatory assertion of the result for optimum time previously stated in section 3.2 of the present study. Total antioxidant capacity, SA and PC clustering close together in both instances (Figure 3a and 3b) is an indication of significant correlation between these factors, while a fixed factor located at PC2 (distance away from variable fixed factors) is an indication that these factors had little or no significant effect on the antioxidant activities studied. Extracting temperature, time and the solid-to-solvent ratio of 60 ºC, 20 minutes and 7.5 w/v were thus established as the optimum antioxidant extracting condition as stated previously in section 3.1 – 3.3.The principal component analysis is a variable reduction tool used to limit factors to fixed numbers of principal components (PCs), which will represent a larger proportion for most of the discrepancy. In a situation where variables are similar, they can be consolidated into a component which can explain most of the variance in the observation. If variables are highly related, they can be unified into a component that can account for the majority of the variances in the observations.

Comparison of hot water extraction with room temperature solvent extraction

Potato peel powder was extracted in organic solvents consisting of acetone, ethanol, methanol, diethyl ether and isobutyl alcohol under similar conditions previously described in this study (see section 3.0), and their extraction yield was compared with hot water extraction. Table 1 shows the optimal yields for water and solvent extraction. Solvent polarity significantly affected polyphenol yield which was in parallel with their decreasing dielectric constant (ε) of each solvent (H_2O, ε = 80.00; MeOH, ε = 33.00; acetone, ε = 21.00; EtOH, ε = 24.55; isobutyl alcohol, ε = 11.00; Et_2O, ε = 4.30). By differentiation, flavonoid, saponins and proanthocyanidin extraction yields were essentially lower in water in respect to organic solvents. Total antioxidant activities were however higher in aqueous extracts in comparison to acetone, diethyl ether and isobutyl alcohol extraction, while polyphenol was found higher in aqueous extracts compare to those obtained when the extraction medium is either acetone or ethanol. Lower dipole moment which prompts lesser dissolvability can be linked to the higher flavonoids, proanthocyanidin and saponins obtained in organic extraction with exception to isobutyl alcohol extraction of proanthocyanidins and acetone extraction of flavonoids.

Table 1 - Effect of various solvents on the extraction yield of potato peel polyphenols and saponins, as well as total antioxidants activities

Bioactive	Levels of bioactives and antioxidant activity					
	Water	Acetone	Ethanol	Methanol	Diethyl ether	Isobutyl alcohol
Polyphenols (mg GAE/g)	5.95 ± 0.02^{d}	1.98 ± 0.02^{f}	4.10 ± 0.03^{e}	17.36 ± 0.03^{c}	30.09 ± 0.27^{a}	27.29 ± 0.46^{b}
Flavonoids (mg CE/g)	1.219 ± 0.004^{e}	1.122 ± 0.019^{f}	2.827 ± 0.002^{a}	1.964 ± 0.002^{c}	1.662 ± 0.005^{d}	1.854 ± 0.002^{b}
Saponins (mg Sap/g)	7.97 ± 0.01^{d}	10.08 ± 0.03^{c}	9.53 ± 0.02^{c}	7.35 ± 0.02^{d}	23.20 ± 0.05^{a}	12.32 ± 0.20^{b}
Proanthocyanidins (mg CE/g)	1.029 ± 0.005^{d}	1.107 ± 0.002^{c}	0.432 ± 0.005^{e}	1.284 ± 0.002^{b}	1.992 ± 0.009^{a}	0.494 ± 0.002^{e}
Total antioxidants (µg Asc/g)	190.83 ± 0.02^{c}	20.17 ± 0.21^{e}	323.73 ± 0.15^{a}	226.77 ± 0.41^{b}	19.02 ± 1.10^{e}	127.29 ± 0.55^{d}

The values are mean ±standard deviations for extractions and those in the same row not sharing the same superscript letter are significantly different from each other (P<0.05)

Polyphenol levels were relatively low in potato peel. There was however increased in polyphenol concentration (30.09 mg GAE/g) when diethyl ether was the extracting solvent, when compared to when isobutyl alcohol or methanol (27.29 and 17.36 mg GAE/g) as the extracting solvents. These values for polyphenol yield were however higher than when water was the extracting solvent when compared acetone and ethanol used as extracting medium. Acetone extraction had the lowest polyphenol level in comparison to other solvents (Table 1). Saponin though regarded as an anti-nutrient by some studies in time past have recently been proven otherwise to be an anti-cancer agent (Guclu-Ustundag and Mazza, 2007; Kadiri *et al.* 2017). Future investigations are encouraged in optimizing this process of saponins extraction for both industrial and clinical applications.

While water had been referred to be safe as the solvent for extraction by several studies and researchers most notably Vuong *et al.* (2013), it is imperative to state that yields of polyphenol, flavonoids and saponins from aqueous extraction were relatively low compared to extraction using an organic solvent which might be a result of the presence of impurities thus warranting further studies on the purification of this compound. However, extraction using the optimum extraction variables lead to considerable improvements in yields of bioactive compounds. It is worthy of note that total antioxidant and radical scavenging activities rightly predicts the level of polyphenol present in the extracts (Table 1). Therefore, a correlation analysis to study the role polyphenol plays in the antioxidant activities of the potato peel was further examined (see section 3.6). The outcomes of this study show that water is an active and efficient solvent for polyphenol extraction from peels of *Ipomoea batatas*. Aqueous extraction will particularly be

useful in the economies of developing countries where capital availability and technical expertise are inadequate.

Correlation between polyphenols and antioxidant activities in potato peels

Prior studies had associated polyphenols derived from plant to have useful health benefits which can prevent the onset of certain forms of cancer, reduce the risk of obesity, diabetics and cardiovascular diseases, and bring about improvement in the functioning of the human immune system (Visioli *et al.,* 2011; Vuong, 2012; Kadiri and Olawoye, 2015). Polyphenols can scavenge free radicals and impeded lipid peroxidation, an association which has been linked to several health benefits (Vuong *et al.*, 2013). Tsao (2010) and Visioli *et al.* (2011) associated their ability to stabilized free radicals by donating an electron to them, thereby neutralizing their potentially damaging chain reactions in cell chemistry while forming stable phenolic products in the process. Notwithstanding their radical scavenging, they are also known as metal chelators which can reduce Fenton reaction rate, thereby preventing oxidation caused by receptive hydroxyl radicals (Tsao, 2010).

To determine the roles which the polyphenol present in the potato peel extracts make to total antioxidants and scavenging activity, a regression analysis was performed (Fig. 4A). The total polyphenol content of extracts obtained under different conditions established to show a linear relationship to the total antioxidant and radical scavenging activities of peel extracts (R_2 = 0.8479 and 0.8305, respectively). This indicates the influence of phenolic compounds on the antioxidant activities study thus far. This result is in agreement with a previous study by Vuoug *et al.* (2014) where a linear correlation (R^2 = 0.87 and 0.84, respectively) describes the relationship between the scavenging and total antioxidant activity of the leaf extracts of *Carica papaya*. Mohdaly *et al.* (2013) had earlier reported significant positive correlation (R^2 = 0.45) between polyphenolic compounds and the antioxidant activity of peel extracts from Marcy and Penta potato varieties.

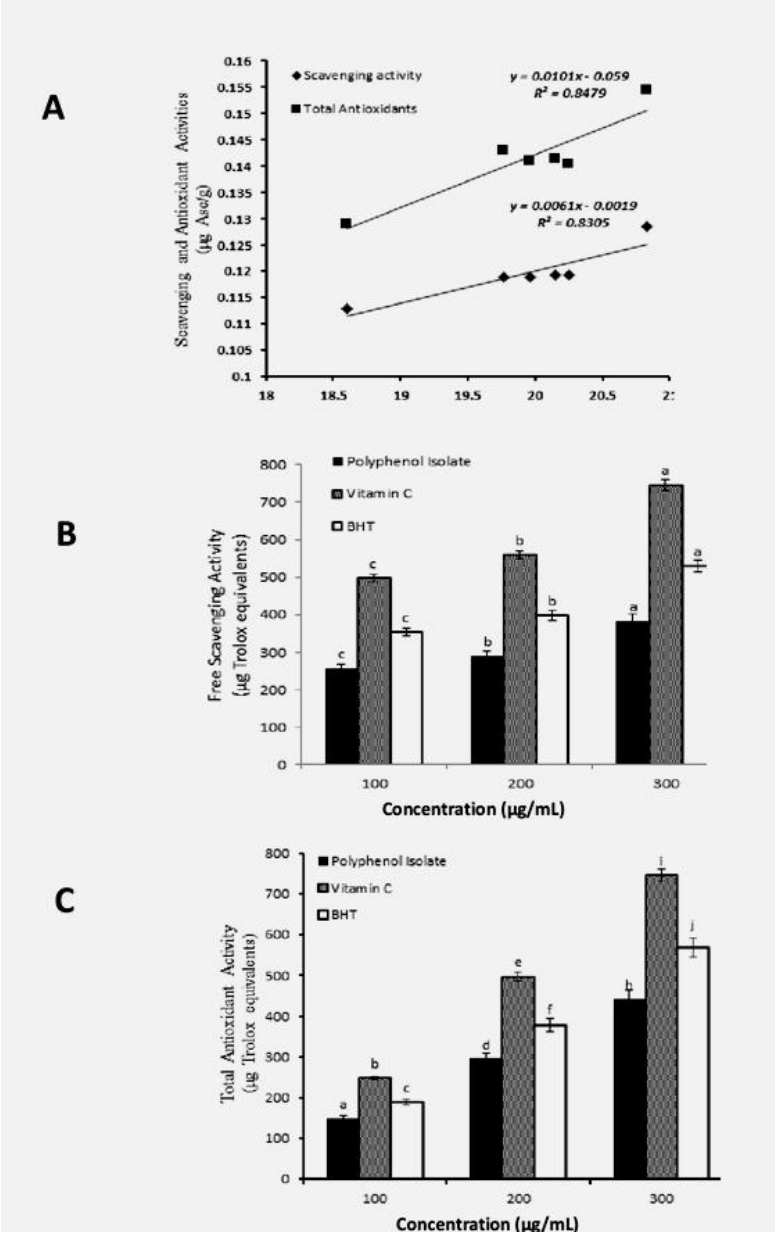

Figure 4 – A: Radical scavenging and antioxidant activity of correlation of polyphenols on free radical scavenging and total antioxidant activities in papaya leaf extract.; (b) DPPH radical scavenging activity; and (c) antioxidant capacities of sweet potato polyphenol isolate, BHT and Vitamin C. The values are mean ± standard deviations and those not sharing a letter on top of the columns are significantly different at P < 0.05.

Preparation of papaya powder, composition and antioxidant activity

To look at the potential medical advantages of potato peel, the antioxidant action of the dried potato peel and polyphenol isolates extracted from it where compared against a scope of standard bioactive compounds, including BHT and vitamin C. The composition of the dried Ipomoea peel and powder are presented in Table 2. The results revealed that an estimated 157.0 g of

polyphenol powder can be gotten from 1kg of dried potato peels by aqueous extraction. Polyphenol content was significantly increased by 243.35 % when dried powder peel powder was refined using a series of processes which includes optimization of polyphenol extraction, an indication that the processes adopted were able to improve polyphenol yield. The same trend was observed for flavonoid content where there was a 342.79 % increase in its recovery. However, there was a slight decrease in the overall yield of saponin. Scavenging and total antioxidants activities increased by 224.12 % and 116.60 % after the refining process of peel powder to polyphenol isolate. These observations were in agreement with a previous study by Amado *et al.* (2014) and Kanatt *et al.* (2005) who reported the peel of potato of their studied varieties to have a high concentration of polyphenol compounds and suggested their use as natural antioxidants.

The improvement in the polyphenol yield in this study might be as a result of the lower temperature (50 ºC) at which evaporation was concentrated which increase the solid content in the polyphenol slurry when compared to the 75 ºC employed by Vuong *et al.* (2013) in their study. The processing conditions study employed in this study has been able to minimize the decomposition of the bioactive compound during its processing from its raw form (peel) to powder (polyphenol isolate).

Table 2: Composition of dried potato peel flour and potato polyphenol isolate powder and their free scavenging and total antioxidants activity.

Parameters	Dried Peels	Polyphenol Isolate
Production Yield (%)		15.7 ± 0.25
Polyphenol (mg GAE/g)	20.25 ± 0.34^{b}	49.28 ± 0.72^{a}
Flavonoids (mg CE/g)	6.45 ± 0.33^{b}	22.11 ± 0.12^{a}
Proanthocyanidins (mg CE/g)	0.80 ± 1.12^{b}	1.26 ± 0.50^{a}
Saponins	6.61 ± 0.34^{a}	5.31 ± 0.25^{ab}
Scavenging activity (µg Asc/g)	34.45 ± 1.25^{b}	77.21 ± 0.22^{a}
Total Antioxidants (µg Asc/g)	188.23 ± 0.10^{b}	219.48 ± 0.35^{a}

The values are mean ±standard deviations for triplicate extractions and those in the same row not sharing the same superscript letter are significantly different from each other ($P<0.05$)

Polyphenol powder extracted from *Ipomoea batatas* peel exhibited significantly reduced total antioxidant and scavenging activities when compared with the commercial antioxidants tested. We proposed this low activity to be a result of the comparatively lower level of polyphenols [15.7 % (w/w)] present in the unrefined powder; a concentration which is approximately 5 times lower than that obtainable in the commercial antioxidant evaluated in this study. Studies are ongoing on enhancing the antioxidant potency of its peel extracts by way of purification.

The antioxidant capacities of the produced polyphenol isolate in the present study were compared with the antioxidant activities of ascorbic acid (vitamin C) and butylated hydroxytoluene (BHT), which are synthetic antioxidant (Fig. 4B). Though the antioxidant activity of the polyphenol isolate produced in the study was slightly lower than those of the synthetic antioxidants, it was however comparable to the antioxidant activities of the commercial antioxidant at a concentration of 200 µg/mL. Slight impurities presence might have impinged the true antioxidant activities in the polyphenol isolate in the current study. Further study is therefore required in obtaining PP polyphenol isolate of 99.9% purity.

Polyphenol isolates extracted from potato peel were also evaluated for their total antioxidant activity (TAC) and compared with the same set of synthetic antioxidants (Vit. C and BHT) earlier discussed (Fig.4C). A dose-dependent trend was observed which was in agreement with a prior study by Vuong *et al.* (2013) who reported a similar trend. Lower antioxidant activities to the studied synthetic antioxidant were observed. Vitamin C was observed to have the most potent antioxidant activities which might be related to purity issue as it is expected that the studies antioxidant compounds have a higher degree of purity compared to out isolated polyphenol isolated in the present study. It was earlier stated that purity issues might be a reason for the significant higher antioxidant reported for Vitamin C when compared to the antioxidant activities of polyphenol isolated from plant parts. The antioxidant activities recorded in this study was, however, higher those reported by Vuong *et al.* (2013) and Amado *et al.* (2014) for *Carica papaya* leaves and *Solanum tuberosum* potato peel respectively.

Conclusion

This study established the optimum processing variables (T, t, E) for the production of polyphenol enrich powder from *Ipomoea* peel using an aqueous solvent as the extracting medium. Optimal extraction conditions were established at 60 ºC, 20 min at a water-to-leaf ratio of 100:7.5 mL/g. These optimum values were validated using multivariate statistical tools including principal component, hierarchical cluster and k mean clustering. Optimal conditions gave rise to a higher yield of polyphenols in comparison to identical mass extractions using acetone and ethanol. The organic solvents nevertheless extracted greater quantities of the antioxidant constituent using certain organic solvents with methanol been the most prominent. Using the principal component analysis, polyphenols in the Ipomoea peels extract were established to be closely correlated with scavenging and total antioxidant activities. About 157 g of powder can be gotten from 1 kg of the peel of *Ipomoea* batata peel through a procedure which was both scalable and reproducible. When compared to commercial antioxidant products such as Vitamin C and BHT, this product can be said to show great potential for application in the food and pharmaceutical industry.

Conflict of interest

The authors declare no conflict of interest

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ORIGINAL RESEARCH ARTICLE

Sustainability in life below water: managing the exploitation of Nigerian shellfish resources

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ABSTRACT
Sustainable Development Goal 14 aims to increase the protection and sustainable management of coastal ecosystems and their resources while addressing threats such as pollution and ocean acidification. Nigeria as a nation with a coastline of approximately 853km facing the Atlantic Ocean is on one hand, at the mercy of the ocean but, on the other hand, the custodian of its resources. These resources like shellfishes sustain us today, and without them future generations will suffer, this is why there is a law against the destructive fishing practices, illegal, unreported and unregulated fishing activities. This article highlights the few priority areas and sustainability challenges for Nigerian shellfish resources as coastal lagoons are fully exploited and increase in harvest of shellfish resources from the lagoons is unlikely. It was noted that despite the availability of regulations, noncompliance by fisher folks has not helped in managing the exploitations. It is therefore recommended that officials need to work with those responsible for social policies, such as employment and other relevant policies, to make sure that the overall government policy direction is coherent and consistent. Also, giving coastal communities access to resources and allowing those access privileges to be traded can help in sustainable management of shellfish resources.

Keywords*: Crustacean, Mollusc, Sustainable development, Ocean resources, Nigeria*.

INTRODUCTION
The Sustainable Development Goals (SDGs) provide multiple opportunities for coastal and marine areas by addressing coastal poverty, prioritizing conservation and explicitly recognising climate change (Friess *et al*., 2019). Coastal and marine environments are relevant to most SDGs, but are explicitly considered under SDG 14, Life below Water: Conserve and sustainably use the oceans, seas and marine resources for sustainable development. SDG 14 aims to increase the protection and sustainable management of coastal and marine ecosystems and their resources while addressing threats such as pollution, coastal habitat destruction and ocean acidification. National policymakers have been criticized for not prioritizing SDG 14 to the same degree as other SDGs (Custer *et al*., 2018). However, SDG 14 was a particular focus at the 2017 High Level Political Forum on Sustainable Development, where 17 out of 43 countries explicitly

stated in their Voluntary National Reviews how they were working towards SDG 14 (UN DESA, 2017).

The coastal and marine focus of SDG 14 means that it may have impacts on production of shellfish resources. As much as forty (40) percent of the ocean is heavily affected by pollution, depleted fisheries, loss of coastal habitats and other human activities (Neumann *et al.*, 2015), the impacts of SDG 14 on sustainability of coastal shellfish resources discussed here are also expected to impact the more than 3 million Nigerians who depend on marine and coastal biodiversity for their livelihoods. The objective of this paper is therefore to offer an insight into the exploitation of shellfish resources for sustainable development in Nigeria.

GLOBAL PRODUCTION OF SHELLFISH

Shellfish is a major component of our global aquatic food supply which includes the molluscs, crustaceans and echinoderms (Moruf and Adekoya, 2018). Crustaceans are invertebrates with segmented bodies, protected by hard shells made of chitin, and include shrimp, lobster, crayfish, crab, and krill. Molluscs are invertebrates with soft bodies, divided into foot and visceral section. They are subdivided into bivalves, cephalopods, and gastropods. The commercially important bivalves are mussels, oysters, clams, and scallops, while cephalopods include squid, cuttlefish, and octopus. The gastropod group contains abalone, sea snail, cockle, and whelks, among others. Echinoderms are not as frequently harvested for food as molluscs and crustaceans, but sea urchin is quite popular in many parts of the world. It is estimated that the ocean is inhabited by more than 1000 species of crustaceans, 50000 species of molluscs, besides 13000 species of finfish (Nybakken, 2001).

Shellfishes are also ideal species to culture because of their low position in the food chain. More importantly shellfish culture is practically a “green” and sustainable industry. This is due to the act of mollusc feeding (biofiltering) which improves water quality by removing particulates (organic matter, nutrients, silts, bacteria, virus etc.) in the water column thus making it an effective way to counter eutrophication (Shunway *et al.*, 2003). The shellfish industry offers great potential to the country in terms of providing food for the people, increasing the income of small-scale fishermen faced with dwindling catches, providing livelihood for people in coastal areas as well as exchange earnings from export of shellfish (Norhana *et al.*, 2011). It is a valuable sub-sector of the fisheries sector, with potentials for local production and export of shellfish products.

According to the State of World Fisheries and Aquaculture, published by the United Nation’s Food and Agriculture Organization (FAO), in 2014, an amount of 167.2 million metric tons (MMT) of seafood was globally available, with landings of shrimp, American lobsters, and cephalopods at 3.5, 0.16, and 4.3 MMT, respectively (FAO, 2016). In recent times, the seafood industry is facing challenges such as concerns about sustainability, slow stagnation of capture fisheries, rising consumer demand, and overall safety of the products. The landing of shrimp, one of the major shellfish commodities, has been stable since 2012 (FAO, 2016). American lobster (*Homarus americanus*) and Norway lobster (*Nephrops norvegicus*) have accounted for more than 60% of world lobster availability, the former reaching a record catch of 160000 tons in 2014. Cephalopods are fast-growing short-lived shellfish; squid is the main component of the cephalopods, followed by cuttlefish, and octopus (Venugopal and Gopakumar 2017; Lawal-Are

et al. 2018). Since 2008, catches of cuttlefishes and octopuses have remained relatively stable at 300000 and 350000 tons respectively (Venugopal and Gopakumar, 2017). The Pacific oyster (*Crassostrea gigas*) is an invasive species most fecund of all oysters while the eastern oyster (*Crassostrea virginica*) is moving progressively toward overfishing (FAO 2016).

Aquaculture production of shellfish has been very limited in West Africa with only Senegal producing Eighty (80) tonnes of the mangrove oyster in 2016 (Gallup *et al*., 2020). The production of shellfish through aquaculture in African region is limited compared with the historical production in Asia, and modern production in Europe and even Latin America (Gjedrem *et al.*, 2012). Except in a few countries, such as Benin, Ghana, and Mauritius, where the attachment of the people to capture fisheries probably enabled them to evolve traditional forms of aquaculture and management (in acadjas, whedos and barochois etc.), aquaculture is not a tradition in most African countries (Alabi, 2010). In developing a viable shellfish culture industry, Nigeria has several advantages including its possession of an extensive coastline with different coastal features such as beaches and mudflats.

THE NIGERIAN COASTAL ZONE

The Nigerian coastline lies on the West Coast of Africa between Latitude 4°10′ to 6°20′N and Longitude 2°45′ to 8°32′E; covering approximately 853 km, from the Seme border in Badagry to Ikang in Cross River State (from West to East) and gently descending into the Atlantic Ocean. Under the Article 57 of U.N Convention on the Law of the Sea (UNCLOS) as ratified in 1994, Nigeria claimed 200 nautical miles as its Exclusive Economic Zone (EEZ), where it has exclusive rights to the exploration and exploitation of all natural resources, in concert with the provisions of the UN Convention on the Law of the Sea. The coastline defines the coastal zone, which is the transition zone between land and water. It is a fragile and biodiverse ecological setting that provides the platform for interaction between terrestrial and aquatic life.

The topography of the Southern Nigerian landscape is low lying, with elevations ranging from 2 to 4 m above mean sea level. It stretches inland to a distance of about 15 km in Lagos to about 150 km in the Niger Delta and about 25 km east of the Niger Delta (Adeaga, 2014). The coastline stretches for about 853km comprising inshore waters, coastal lagoons, estuaries and mangrove, characterised by periodic tidal variations and ranges along water channels and the differences depend on the hydrological properties and slopes of the various channels (Zabbey *et al*., 2019). The most commercially important living resources are fin and shellfishes including shrimps, predominantly penaeid shrimps. Fishing is a major activity especially in the coastal areas where important shellfish resources are obtained.

NIGERIAN SHELLFISH RESOURCES

According to the Fisheries Statistics of Nigeria, the estimated potential yield for shellfishes is 51,760 metric tonnes, while the most produced shellfishes are the shrimps and the prawns (FDF, 2007). Nigeria has an important shrimp trawling industry harvesting mainly pink shrimp (*Farfentepenaeus notialis)*; although reliable production data is scarce while historical data reports landings of between 10,000 and 15,000 metric tonnes annually (FDF, 2007). Discrepancies are usually accounted for by illegal at-sea sales that go unreported. According to Zabbey (2006), a total of 173 licensed vessels are trawling for shrimp in Nigeria. Overall production of fin and shellfish increased from 1995 to 2006 and slightly decreased subsequently

due to problem of militants and piracy in marine waters (Olaoye and Ojebiyi, 2018). However, basic data may not be available to judge if the resources are exploited sustainably and scientifically. At least the coastal prawn fisheries are under the heavy pressure of fishing activities (Nakazawa *et al*., 2013).

The pink shrimp (*Farfentepenaeus notialis*) has been the dominant target and supportive species in Nigeria (Alabi, 2010). Prior to the end of the 20th century, *F. notialis* fishery was quite lucrative, resulting in bumper harvest by trawlers. Perhaps, the licensing of vessels without ensuring that trawl owners respect the state of existing stocks culminated in the collapse in *F. Notialis* fishery which resulted in the winding or withdrawal of some trawlers from Nigeria around 2000; then, industrial shrimping was no longer profitable as before but thanks to the sudden emergence of *Peneaus monodon*, an alien species which has revived or prevented industrial shrimp operations from total collapse in Nigeria (Jimoh and Lemomu, 2010). The economically important shellfish resources in Nigerian inshore and offshore waters are shown in Table 1

Table 1: **Economically important shellfish resources in Nigerian Coastal waters**

Crustaceans	Moluscs
Shrimps	**Bivalves**
Pink shrimp (*Farfentepenaeus notialis,* Pérez Farfante 1967)	Mangrove oyster (*Crassostrea gasar*, Adanson, 1757)
Guinea shrimp (*Holthuispenaeopsis atlantica*, Balss 1914)	West African Clam (*Galatea paradoxa*, Born, 1778)
Red deep-water shrimp (*Parapenaus longirostris*, Lucas 1846)	Ark clams (*Anadara senilis*, Linnaeus, 1758)
Stripped or tiger shrimp (*Penaeus monodon*, Fabricius 1978)	Blue mussel (*Mytilus edulis*, Linnaeus, 1758)
Prawns	Cockles (*Cardiumcostatum*, Linnaeus, 1758)
Estuarine prawn (*Nematopalaemon hastatus*, Aurivillius 1898)	Donacid clams (*Egeria radiate*, Larmack, 1804)
African river prawn (*Macrobrachium vollenhovenii*, Herklots 1851)	**Gastropods**
Brackish water prawn (*Macrobrachium macrobrachion*, Herklots 1851)	Edible periwinkle (T*ympanotamus fuscatus*, Linnaeus, 1758).
Gabon Shrimp (*Atyagabonensis*, Giebel 1875)	Periwinkle(*Pachymelina aurita*, Linnaeus, 1758)
Lobsters	Murid snail (*Thais haemastoma*)
Spiny lobsters (Royal Spiny Lobster, *Panulirus regius*, De Brito Capello, 1864)	Other snails like *Biomphalaria pfeifferi, Biomphalaria globosus* and *Lymnaea natalensis*
Locust lobsters (*Thenusorientalis*, Lund, 1793)	
Crabs	
Swimming crabs (*Callinectes amnicola*, De Rochecbrune, 1883 and *Portunus validus*, Herklots, 1851)	
Deep sea crabs (*Chaceonatopus*, Manning &Holthuis, 1989)	
Land-based crabs (*Cardiosoma armatum* Gecarcinidae, *Sesarmahuzardi* and *Goniopsispelii* Grapsidae and *Ocypoda africana* Ocypopidae).	

SUSTAINABILITY CHALLENGES FOR EXPLOITATION OF NIGERIAN SHELLFISH RESOURCES

Environmental challenges

- The changes in the physical aspects such as temperature, and the biological aspects such as species competing for the same food supply, mean that many influences on the number of shellfish available in a given year are outside of human control, and thereby difficult to manage. For example, swimming crab populations go through a regular cycle every decade which makes fish farmers to adjust the fishing activity regularly in Nigeria.

- Shellfish stocks vary depending on naturally occurring conditions in marine ecosystems and in the linked lagoon and estuaries. Studies now point to climate change as driving physical and biochemical shifts in the ocean environment at the global scale. The ocean is rising and, overall, is becoming warmer and more acidic. While some stocks may benefit from warmer water, these shifts may threaten the ecological viability of other stocks and may make it more difficult to estimate shellfish populations.

- Shellfish stocks may also be harmed by other human activities that take place in the ocean, such as offshore oil and gas exploitation. Activities on land have also degraded shellfish stocks in our lagoons, for example, runoff of sediment from agricultural land.

- Coastal pollution as a result of high level of population in Nigeria. There has been an increased application of technologies in the exploration and exploitation of natural resources aimed at producing more food, goods and services. The rise is industrialization and agricultural activities have led to release of different categories of wastes into the environment. These wastes sometime reach and exceed toxic level and are thus classified as pollutants. In Nigeria, the developments of the petroleum industry release pollutants such as petroleum hydrocarbon into the coastal environment. Solid wastes from domestic homes, sewage and effluents from industries also pollute the marine environment and have adverse effect on the shellfish resources (Usese *et al*., 2019)

Economic challenges

- Some shrimpers operate globally and market their products around the world. As a result, they may be affected by international economic trends and events that include increases in the price of fuel, the impact of foreign exchange rates on export markets, offshore oil spills in other countries; in particular, the economic context for shellfisheries is shifting as a result of the global interest in eco-certification.

Social challenges

- Management of shellfish resources has indirect effects on the processing industries that handle the products and on the coastal communities where the fishers and their families live. Because these communities rely socially and economically on fishing incomes, official may feel political pressure to increase harvest quotas, even when the stocks may be at risk. Or communities may seek to have more of a say in how the fishery is managed or how access to the resource is distributed.

- Fishing in the non-trawling zone: Most often than not, trawlers operate beyond permissible limits (within non-trawling zone) and disregard other polices such as recommended mesh sizes of net. Some trawl captains desirous to make good catches, oftentimes trawl in the 5 nautical miles non-trawling zone. This unscrupulous fishing activity could result in avoidable conflicts between local fisher folks and their industrial counterparts.

Organizational challenges

- Inadequate information may result in overexploitation of shellfish stocks or could lead to missed opportunities for economic benefits.

- Lack of exploratory data for effective stock management. The paucity of data on shellfish stocks inevitably warrant the over dependency on precautional approach as the only management option in Nigeria. Worst still, the lack of transparency in catch data reporting, transhipment at sea and negligence on the part of the regulatory bodies with regard to data collection have made it near impossible to know precisely how much shellfish is taken from the natural stocks daily, monthly or annually (Zabbey, 2007)

- Bycatch and discard problem in Nigeria shrimp trawl fisheries result to multitude of species presupposes to high bycatch and discard rates (Eayrs, 2005). FAO has recently estimated that nearly 7 million tonnes of fish bycatch is discarded globally by commercial fishermen every year.

SUSTAINABLE EXPLOITATION OF SHELLFISH RESOURCES

Sustainable development is the management and conservation of the natural resources and the orientation of technological and institutional change in such a manner to ensure the attainment and continued satisfaction of human needs for present and future generations (FAO, 1995). The basic principle that governs sustainable development of fisheries is that, it must be conducted in a manner that does not lead to over-fishing, or for those stocks that are over-fished; the fishery must be conducted such that there is a high degree of probability that the stock(s) will recover and also fishing operations should be managed to minimize their impact on the structure, productivity, function, and biological diversity of the ecosystem (Joshi *et al*., 2018). The general stagnation in marine fish production during the last decade gives rise to concern about the sustainability of Nigerian shellfish resources.

Sustainable management of shellfish resources cannot be achieved without an acceptance that the long-term goals of fisheries management are the same as those of environmental conservation. Global wild fisheries are believed to have peaked and begun a decline, with valuable habitats, such as estuaries and coral reefs, in critical condition (Tietenberg, 2006). Sustainable shellfisheries development can be achieved through responsible fishing, which considers rational fishery management objectives that address a range of issues including the status of the resource, the health of the environment, post-harvest technology and trade, as well as other economic concerns, social benefits, legal and administrative support. In the case of shared resources, a co-ordinated approach to responsible fisheries management is essential, and Caddy and Griffiths (1995) proposed the following actions:

- **Regulate fishing effort**: It is crucial to control fishing effort, and to avoid financial incentives that would contribute to excess fishing capacity. Excess fishing capacity and overcapitalization threaten the sustainability of the resource and its industry.

- **Establish code of conduct for responsible fishing to guide management plan**: This is needed to maximize benefits while avoiding wastage caused by indiscriminate fishing practices such as harvesting of undersized shellfish and non-target species, and to avoid use of gears that have a negative impact on the environment.

- **Establish and support international fishery commissions and organisations concerned with management of shared resources**: The relevant international fisheries agreements promote participation in, and financial support for, the work of these commissions and organisations.

- **Regular consultation among harvesting countries**: Parties sharing the resources need to consult and collaborate regularly so as to promote understanding and full cooperation.

- **Set agreed management objectives and related reference points, incorporating a precautionary approach**: Agreement on management reference points during the early stages of the fishery will help to ensure full cooperation of participants with management decisions. Where there is scientific uncertainty, a precautionary approach to management is recommended.

- **Develop contingency plans**: Management plans should incorporate some contingency for dealing with sudden and unpredictable environmental changes caused by man-made or natural disasters.

- **Develop mechanism for resolving user conflicts**: Management should provide mechanisms for handling problems arising from resource user conflicts.

- **Protect biodiversity**: The biodiversity or species richness of an ecosystem is an important measure of ecosystem health. The preservation of biodiversity will ensure that present human development activities do not threaten the ability of future generations to meet their own needs.

- **Protect the environment**: There should be monitoring and control of waste disposal and pollution. In addition, every effort should be made to prevent discarding of entangling material that could trap and kill species or physically damage the environment.

- **Promotion of research**: Research should be conducted to support and inform various aspects of management.

- **Optimise social and economic stability**: There should be fair and equitable distribution of benefits derived from the shellfishery.

Other key principles to help achieve a sustainable exploitation of shellfish resources in Nigeria are identified as:

- **Effective governance arrangements provide a foundation for sustainability**: The Food and Agriculture Organization has concluded that fisheries are at greatest risk when governance is weak or absent. If governance arrangements are good, then preferred policy outcomes are more likely to be achieved.

- **Include stakeholders in the planning process**: Stakeholders can take part through data collection, knowledge gathering, collaborative research, option analysis, decision making, and other aspects of running the shellfishery. In this case, fisheries management decision-making processes will be seen to be fair, transparent and subject to clear and consistent rules and procedures.

- **Establish clear lines of accountability**: This includes clear roles and responsibilities, clear performance expectations, expectations balanced with capacity, credible reporting, and reasonable adjustment and review.

- **Evaluate whether objectives have been achieved**: Managers can evaluate their success by drawing on the information collected from scientific studies, monitoring, and surveillance, and by using the information supplied by fishers themselves. It will give hint whether a shellfish stock is being fished sustainably.

CONCLUSION

The goals of SDG 14 and sustainability in shellfish resources can contribute to achieving SDG 2 (Zero Hunger) due to their important role in the food security of coastal communities. A push to achieve SDG 2 will indirectly impact fisheries. Coastal resources such as shellfish resources are strongly linked to poverty and development since they provide food security to potentially millions of Nigerians. An SDG 14 focused on coastal and marine ecosystems – the life below water – is therefore encouraging. However, a stronger recognition of the unique challenges of coastal lagoons/sea, throughout most SDGs may raise its profile so that they can be more strongly considered in conservation and development of its resources like shellfishes. Sustainable shellfisheries development can be achieved through responsible fishing, which considers rational fishery management objectives that address a range of issues including the status of the resource, the health of the environment, post-harvest technology and trade, as well as other economic concerns, social benefits, legal and administrative support. In the case of shared resources, a co-ordinated approach to responsible fisheries management is essential.

RECOMMENDATIONS

Those responsible for managing fisheries need to try to understand how shellfish stocks will be affected by natural or environmental disasters and recognize the cumulative pressures on the stocks that they manage. They also need to work with those responsible for social policies, such as employment policies, to make sure that the overall government policy direction is coherent and consistent. Officials should ensure that fishers comply with legal and policy requirements

Giving fishers or coastal communities' access to resources and allowing those access privileges to be traded can help to reduce overfishing. Fisheries managers need processes for deciding

which shellfish will be caught, how many will be taken, by whom, where, when, and using what equipment. These decisions are among the most powerful tools available to managers to influence the direction and sustainability of the shellfishery.

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ORIGINAL RESEARCH ARTICLE

Synthesis, characterization and biological evaluation of hexagonal wurtzite structured ZnO nanoparticle from Zn (II)-Schiff base complex

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Abstract

Studies on transitional metal compounds of Schiff base ligands have been of great significance due to their spectral properties and wide applications. Tetradentate Schiff base-Zn (II) complex was prepared and used as a precursor for the synthesis of Zinc oxide (ZnO) nanoparticles through **a** one-step calcination process at a different temperature: 250-350 °C. The influence of temperature on the antioxidant activities of synthesized nanoparticles was investigated. The precursor (zinc complexes) was characterized by melting point, thermogravimetric analysis, UV-Vis, FT-IR spectroscopy. elemental analysis, and thermogravimetric analysis (TGA) of tridentate Schiff base was synthesized and characterized using The structural studies of synthesized metal oxides were carried out with powder X-ray diffraction (PXRD), transmission electron microscopy (TEM), FTIR, and UV-visible. The energy band gap of the nanoparticles was 3.15 eV for ZnO@250, 3.31 eV for ZnO@300, while ZnO@350 was found as 3.17 eV and 3.56 eV. The average sizes of the ZnO nanoparticles were found to be around 25 nm. The antioxidant activities of the product were investigated through scavenging activity on DPPH. The obtained IC_{50} value of the DPPH activity for the product @ 350 °C (IC_{50} = 4.09 ± 0.32µM) was higher than other nanoparticles.

Keywords: *Schiff base, Zinc Oxide, DPPH, Metal complexes, Antioxidant, Nanoparticles*.

Introduction

Metal complexes containing Schiff base are of interest because of their electronic properties that can be modified by suitable functionalization with amine and cyclic substituents (Ejidike and Ajibade, 2017; Ejidike, 2018). Also, tetradentate Schiff base complexes have shown high stability when coordinated to metal ion using the N_2O_2 donor atoms (Alias *et al.*, 2014, Emara *et al.*, 2014; Ejidike and Ajibade, 2017). There have been current advances primarily with the capability to prepare highly ordered nanoparticles of assorted shapes and sizes, in the field of

nanotechnology, and this has led to the development to new materials possessing various biological activities (Meruvu *et al.*, 2011; Gunalan *et al.*, 2012; Navale *et al.*, 2015; Stan *et al.*, 2016).

Metal oxide nanoparticles can be obtained by various methods depending on the chemical and physical techniques employed. Metal oxide nanoparticles and composite materials are widely applied in the field of research and development and diverse applications in industries including surface coatings, bioengineering, bio-diagnostics, optoelectronics, and agriculture (Navale *et al.*, 2015; Malathy *et al.*, 2017). Zinc oxide nanoparticles (ZnO NPs) have received considerable attention due to their unique antibacterial, antifungal, and UV filtering properties, high catalytic and photochemical activity (Stan *et al.*, 2016). Among the metal oxide nanoparticles, zinc oxide is interesting because it has vast applications in various areas such as optical, piezoelectric, magnetic, and gas sensing (Navale *et al.*, 2015; Stan *et al.*, 2016; Xaba *et al.*, 2016). Besides these properties, ZnO nanostructure exhibits high catalytic efficiency, strong adsorption ability and are used more and more frequently in the manufacture of sunscreens (Meruvu *et al.*, 2011; Kumar *et al.*, 2014; Navale *et al.*, 2015), ceramics and rubber processing, wastewater treatment, and as a fungicide (Stan *et al.*, 2016; Navale *et al.*, 2015). Nanostructures based on zinc oxide are predominantly interesting because of their n-type conductivity with a wide band gap of 3.3 eV, making ZnO materials more suitable for modern technologies (Kalpanadevi *et al.*, 2013; Kulkarni and Shirsat, 2015; Xaba *et al.*, 2016).The significance of ZnO NPs in various areas has led to its global interest in studying their antifungal, antibacterial, antioxidant, and anticancer activity. Literatures on the biological activities actions of ZnONPs have stimulated a considerable range of antimicrobial and toxicity applications. ZnO NPs are used as antibacterial agents owing to the unique properties and excellent stability with long life as compared to organic-based disinfectants (Kumar *et al.*, 2014; Stan *et al.*, 2016). The large surface-area-to-volume ratio allows their use as novel biological agents. This unique property has also predicted to enhance ZnO NPs applications in several areas, such as in catalysis and biomedicine, food industry (Meruvu *et al.*, 2011; Kumar *et al.*, 2014; Stan *et al.*, 2016)

Reactive oxygen species (ROS) or free radicals are products of the in vivo physiological and biochemical processes in the living cells (Ejidike and Ajibade, 2017) that could cause lipid peroxidation and may lead to diseases such as cancer, cardiovascular diseases, immunodeficiency, liver injury and other infections (Ejidike and Ajibade, 2015; 2017). To safeguard the human body against cell damage by oxidative species, antioxidant is of paramount importance (Ejidike and Ajibade, 2015). Due to many biological processes taking place at the nanoscale level, there is the potential that engineered nanomaterials may interact with biomolecules and cellular processes; hence, ZnO nanoparticles are believed to be safe, non-toxic, and biocompatible. Based on the above facts in the present work, we report the synthesis and characterization of Zn(II) Schiff base complexes and its ZnO nanoparticles. Also, reported are the antiradical activities of the synthesized nanomaterials.

Experimental

Materials

All chemicals and solvents were of analytical grade and used as obtained without any further purification. Ethylenediamine and the zinc chloride acetate were purchased from Merck, South Africa; 2',4'-dihydroxyacetophenone, Ascorbic acid, gallic acid, and 2-hydroxybenzaldehyde

were purchased from Sigma-Aldrich (Johannesburg, South Africa); 1,1-diphenyl-2-picrylhydrazyl (DPPH) was purchased from Sigma Chemical Co., USA. Perkin Elmer FT-IR spectrometer (Spectrum 2000) in the range 4000–400 cm^{-1} was used for IR spectra data collection. A freshly prepared 10-3 M DMF solution with PC 7000 conductivity cell was used for the conductivity measurements. The analyses of carbon, hydrogen, and nitrogen were determined on a Perkin Elmer elemental analyzer (2400 Series). Electronic spectra were recorded on a model T80+ UV-Vis spectrometer in the range 200–800 nm. Thermal Decomposition of the Complexes were recorded on the Thermogravimetric analyzer: TGA 4000 System. SMP 10 Melting Point Apparatus was used for the melting points analysis. Transmission electron microscopy (TEM) studies were performed using a JEOL JEM-2100F TEM operated at an accelerating voltage of 200 kV.

Preparation of Schiff base ligand

The ligand (L) was prepared according to the previous report (Ejidike, 2018).1,2-ethylenediamine (0.015 mol) dissolved in 30 ml of ethanol was slowly added to an ethanol solution (30 ml) containing 2-hydroxybenzaldehyde (0.015 mol), followed by the slow addition of 2',4'-dihydroxyacetophenone (0.015 mol) dissolved in 30 ml ethanol. The resulting coloured mixture was refluxed with stirring for 4.5 h. It was cooled and the resulting precipitate was filtered, washed with ethanol, and then recrystallization in ethanol (Yield = 91.20 %).

Preparation of the Precursor [Zn-L] complex

In a typical experiment, the metal complex was obtained by adding 0.01 mol of $Zn(acet)_2 \cdot 2H_2O$ dissolved in 30 ml of ethanol, into a warm ethanolic solution (40 ml) of (0.01 mol) ligand (L)with constant stirring. The resulting solution was then refluxed for 3 h and allowed to cool to room temperature. The solid formed was filtered, and the precipitate was carefully washed with cold ethanol and diethyl ether, dried over dry calcium chloride, and characterized (Scheme 1).

Ligand ($C_{17}H_{18}N_2O_3$): The ligand was obtained as a yellowish solid. Percentage yield (%): 91.20. Decomp. Temp.: 198-199 °C. Formula weight: 298.34 g. Anal for $C_{17}H_{18}N_2O_3$calcd.: C, 68.44; H, 6.08; N, 9.39 %. Found: C, 67.91; H, 6.74 N, 8.98 %. FT-IR spectral bands (cm^{-1}): ν(OH), 3365; ν(C=N), 1582.UV-vis (λ_{max}, nm): 295, 320, 400.

Zn($C_{17}H_{16}N_2O_3$) complex: The complex was obtained as a lemon-yellow solid. Percentage yield (%): 73.28. Decomp. Temp.: 225-226 °C. Formula weight: 361.73 g. Anal for $C_{17}H_{16}ZnN_2O_3$calcd.: C, 56.45; H, 4.46; N, 7.74 %. Found: C, 57.01; H, 5.04 N, 7.49 %. Conductivity (µS/cm): 14.15. FT-IR spectral bands (cm^{-1}): ν(OH), 3399; ν(C=N), 1589; ν(M-O), 626; ν(M-N), 437. UV-vis (λ_{max}, nm): 295, 325, 410.

Preparation of Zinc Oxide Nanoparticles

Zinc oxide nanoparticles were obtained by the Calcination method (Malathy *et al.*, 2017) of the precursor. In this method, the dried precursor (Zinc Schiff base complex) was transferred to a silica crucible and heated in an electric furnace at various temperatures such as 250 °C, 300 °C and 350 °C (Table 1). in an ordinary atmosphere for about 1 h. The precursor started decomposing gradually, and the total decomposition of the precursor complex led to the formation of the ZnO nanoparticles, which are quenched to room temperature, and stored in a desiccator. The pure ZnO NPs obtained were characterized by spectral studies (Scheme 2).

Table 1: Codes used for the samples descriptions

Codes	Description
ZnO@250	ZnO nanoparticles prepared at 250 ℃
ZnO@300	ZnO nanoparticles prepared at 300 ℃
ZnO@350	ZnO nanoparticles prepared at 350 ℃

Biological Evaluations

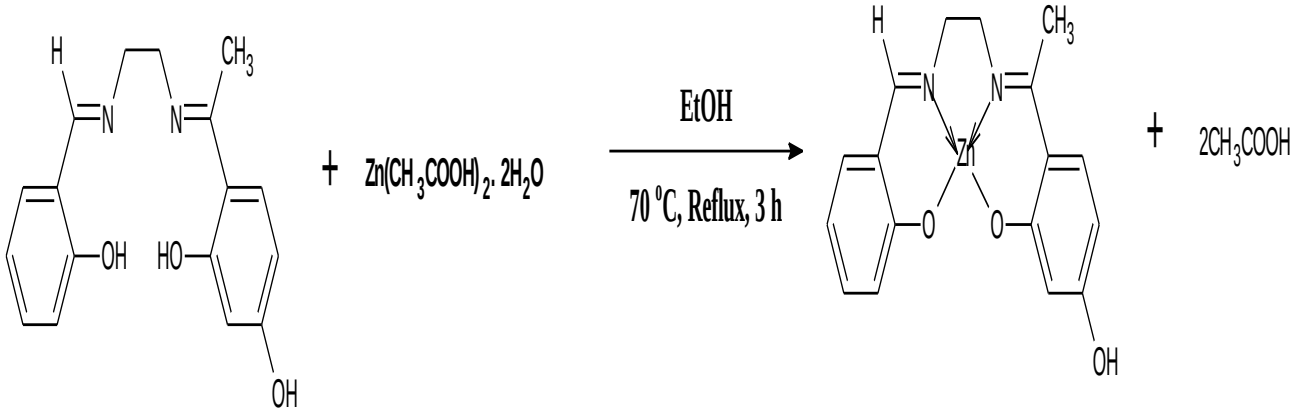
2,2-Diphenyl-1-picrylhydrazyl (DPPH) Radical Scavenging Activity

DPPH (1,1-diphenyl-2-picryl-hydrazyl) radical scavenging activity evaluation is a standard assay used in antioxidant activity studies. The antioxidant activity of the ZnO nanoparticles was studied spectrophotometrically by the DPPH method (Ejidike and Ajibade, 2017). It is a rapid technique for screening the radical scavenging activity of synthesized compounds (Ejidike, 2018). The radical scavenging properties of the synthesized zinc oxide nanoparticles with DPPH radical were assessed at different concentrations of the test compounds in DMF solutions (1 mL), added to 1.0 mL of 0.4 mM DPPH in methanol and mixed thoroughly by the vortex. The mixtures were allowed to incubate at room temperature in the dark for 30 min, after which the scavenging power of the test samples was measured concerning a decrease in the absorbance of DPPH at 517 nm. All tests analysis was performed in triplicates, to obtain the mean ± SD.

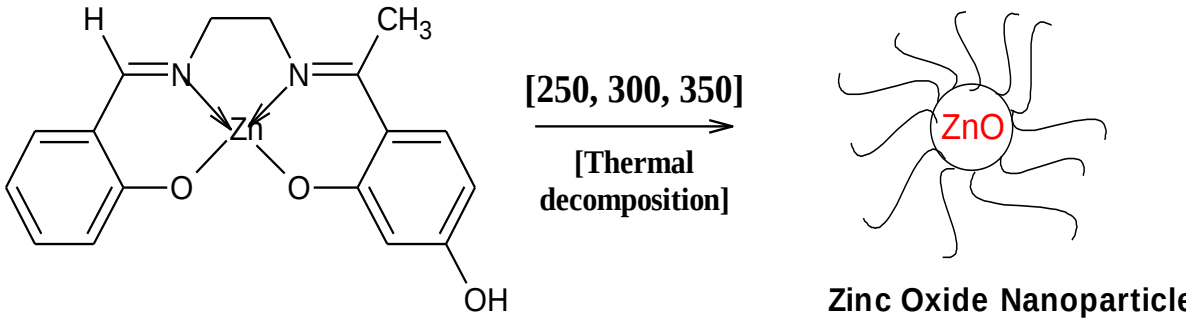
$$\% \text{ scavenging activity} = \frac{\boldsymbol{Absorbance\ of\ control - Absorbance\ of\ sample}}{\boldsymbol{Absorbance\ of\ control}} \text{ X } 100 \qquad (1)$$

Results and Discussion

The metal complex was obtained by the reaction of zinc acetate in methanol with the ligand in a 1:1 mole ratio. The complex analytical data disclose metal: ligand molar ratio (1:1) for the system (Scheme 1). The complex was soluble in DMF, DMSO, and insoluble in other organic mediums, with molar conductivity measured in DMF solution indicating a non-electrolyte character.



Scheme 1: Reaction route for the preparation of the metal complexes



Scheme 2: Reaction route for the preparation of the metal oxide nanoparticles (250, 300, 350 = 250°C, 300°C, 350°C)

FTIR Spectra

A band around 3365 cm^{-1} is assigned to ν(OH) stretching vibrations of the free Schiff ligand. In the complex spectra, this band shifted to ~3399 cm^{-1} owing to the ν(OH) stretching of the moisture content within the complex (Alias *et al.*, 2014, Emara *et al.*, 2014; Ejidike, 2018). The intense band observed at 1582 cm^{-1} in the free ligand assigned azomethine ν(C=N) stretching vibration (Ejidike and Ajibade, 2017) was shifted toward higher wave number in the metal complex spectra (Figure 1). This shifting towards higher wave number around 1589 cm^{-1} regions is due to the coordination of the azomethine (>C=N) nitrogen atoms with the central metal ion (Malathy *et al.*, 2017; Ejidike, 2018). The stretching vibration of the phenolic ν(C-O) observed at 1240, and 1170 cm^{-1} in the free Schiff base (Kumar *et al.*, 2014; Ejidike, 2018) undergo a hypochromic shift to 1243 and 1173 cm^{-1} regions in the zinc complex upon complexation. This shift further confirms the coordination of the phenolic oxygen leading to the formation of the C-O-Zn bond (Ejidike and Ajibade, 2017). New bands observed around region 626 cm^{-1} in the complexes are assigned to ν(M-O) stretching vibrations while those in the region 437 cm^{-1} are due to ν(M-N) (Ejidike and Ajibade, 2017; Malathy *et al.*, 2017).

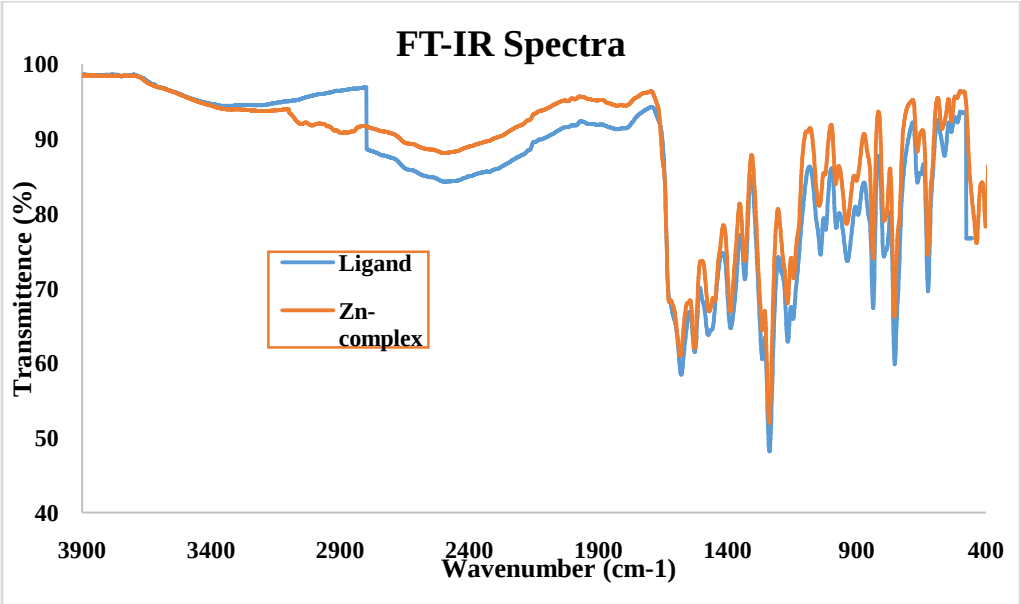


Figure 1: FT-IR for Ligand (L) and complex (Zn-L)

UV-vis Spectra

The UV-visible spectra of the free ligand (L) and the metal complex [Zn-L] are shown in Figure 2. The ligand (L) spectra show bands in the region 295 and 320 nm which is attributed to π-π* for the aromatic system, while the third band is due to n-π* transition within >C=N group. The π-π* transition was shifted to a corresponding wavelength 295 and 325 nm upon the formation of the zinc complex (Figure 2). The wavelength observed at 400 nm in the UV – Vis spectra of the ligands (L) corresponding to the n-π* transition was shifted to 410 nm in the complex upon complexation, this is an indication of the coordination of ligand to zinc ion (Alias *et al.*, 2014, Ejidike, 2018). The Zn(II) complex, showed an absorption band at 410 nm is due to the LMCT/ MLCT transitions of zinc complex in a tetrahedral geometry, as no d-d electronic transition isexpected (Malathy*et al.*, 2017; Ejidike, 2018).

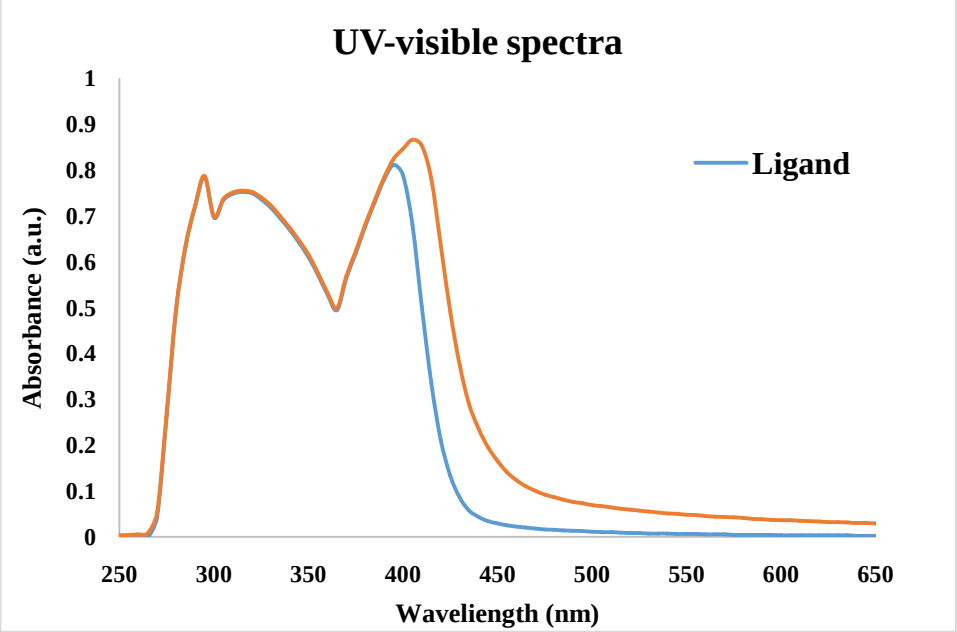


Figure 2: UV-Vis for ligand and Zn (II) complex

Thermogravimetric analysis (TGA)

Thermogravimetric and derivative thermogravimetric analysis (TGA/DTA) of the synthesized zinc-Schiff base complex was measured under a nitrogen atmosphere at a heating rate of 10 °C min^{-1} from 20 °C to 900 °C. TG/DTG results were plotted as % weight loss against temperature; provides insight into nature, properties of different molecules, and the residues obtained after thermal decomposition. The loss of lattice water and part of the anchoring ligand in the zinc-complex occurs between the temperature ranges of 134 – 255 °C. Above this temperature, as displayed in Figure 3, it revealed a strong endothermic peak in the range of 297 – 396 °C for the as-synthesized complex. The differential thermogravimetric curve illustrates that most of the weight loss occurred at about 319 °C. It exhibited a sharp decomposition with a mass loss of 55.02 %, which is assignable for the loss of the ligand moiety, which gives zinc oxide as the final residue (Kalpanadevi*et al.*, 2013; Malathy*et al.*, 2017; Ejidike, 2018).

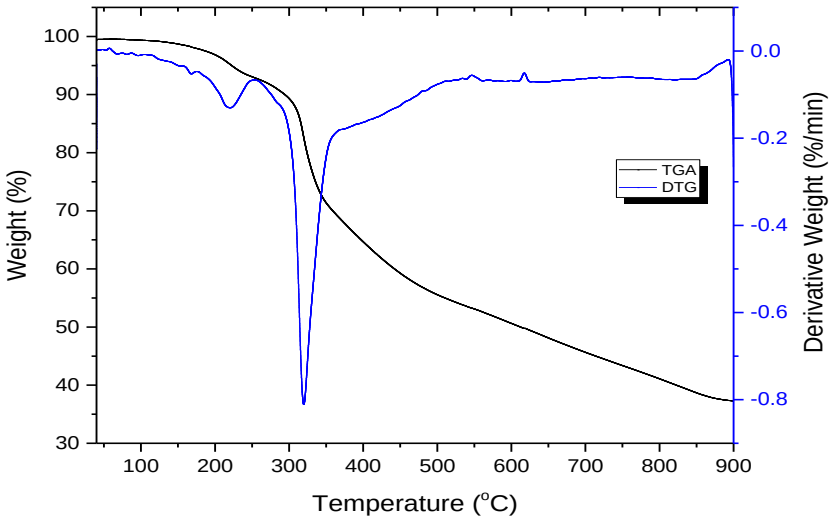


Figure 3: TG and DTG curves of Zn (II)-complex

Characterization of Zinc Oxide Nanoparticles

FTIR Spectra for ZnO

The FTIR spectra of the ZnO Np's synthesized from the complex, shows ν(Zn-O) vibrations at 761 cm^{-1}.The presence of the O-H group represents the presence of water molecules on the surface of ZnO nanoparticles (Kalpanadevi*et al.*, 2013; Stan *et al.*, 2016; Ejidike, 2018).

Optical Properties of Zinc Oxide Nanoparticles

The absorptions spectra of the zinc oxide nanoparticles are presented in Figure 4a. UV-Visible spectra analysis has served as a useful technique for the characterization of semiconductor nanoparticles, which exhibit quantum size effect. The UV-Vis absorption spectra (Figure 4) showed that the absorption peaks at 382 nm for ZnO@250, 380 nm for ZnO@300, and 306 and 336 nm for ZnO@350 of the analyzedZnO particles, which are relatively blue-shifted of the bulk ZnO materials (Kalpanadevi*et al.*, 2013; Xaba*et al.*, 2016; Gharibshahi*et al.*, 2017). The band gap of the ZnO nanoparticles were calculated by extrapolating the curve drawn between the square of (αhν) versus (hν) as displayed in Figure 4b. Where ν is the frequency and α is the optical absorption coefficient. Therefore, the band gap energy was obtained by extrapolating the curve and found to be approximately 3.15 eV for ZnO@250, 3.31 eV for ZnO@300, while ZnO@350 was found as 3.17 eV and 3.56 eV.The blue shifts of the ZnO nanoparticles are indicative of quantum confinements of the nanoparticles (Kulkarni and Shirsat, 2015).

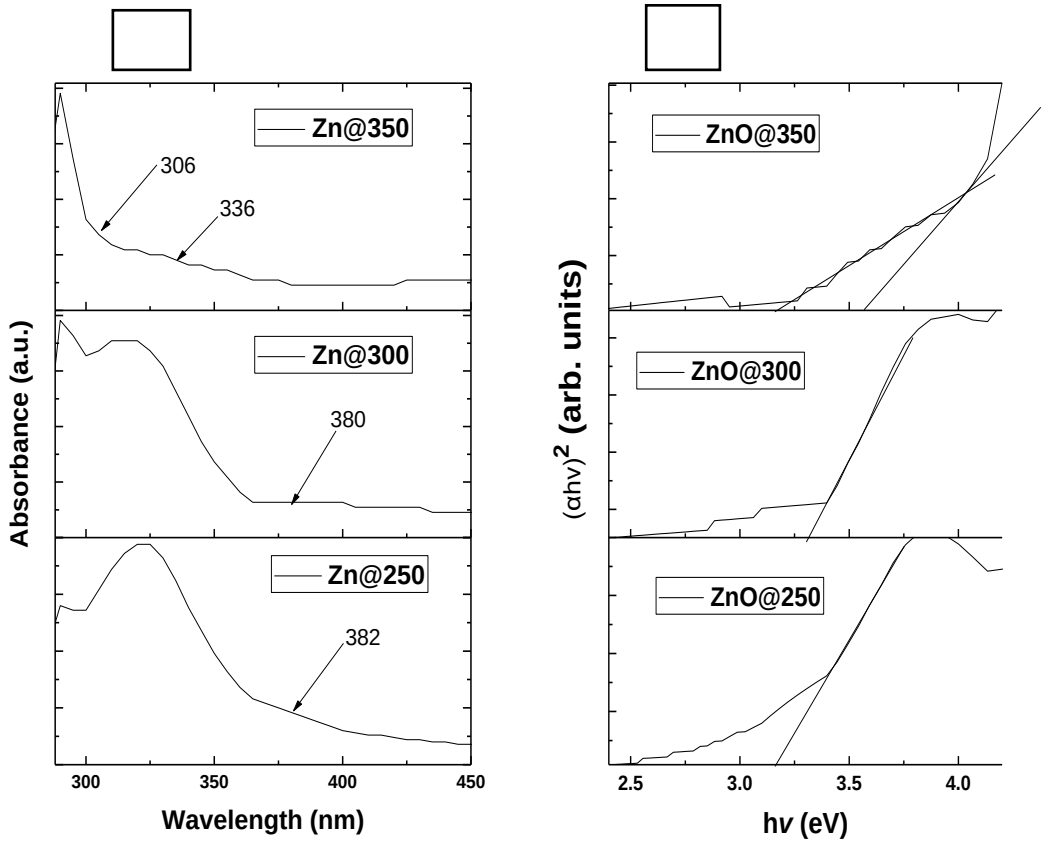


Figure 4: (A) UV-Vis spectra of the nanoparticles; (B) Tauc Plot from UV-Vis spectra for band gap determination of as-synthesized ZnO nanoparticles

X-ray diffraction studiesof ZnO nanoparticles

The X-ray diffraction peaks of ZnO nanoparticles are shown in Figure 5. The positions of XRD peaks observed in the patterns of the synthesized ZnO nanoparticles corresponds to the planes (100), (002), (101), (102), (110), (103), (202), (112), and (201) showing a good agreement with those of the hexagonal wurtzite structure for bulk ZnO with lattice constants of a = 0.3 249 nm and c = 0.5208 nm (JCPDS card No. 36-1451, P63mc) (Navale*et al.*, 2015; Kulkarni and Shirsat, 2015). The average crystallite size (D) of the Zn-NPs is calculated after appropriate background corrections from X-ray line broadening of the diffraction peaks using Debye Scherrer's formula

$$D = {}^{0.9\lambda}/_{\beta \cos\theta} \qquad (2)$$

where λ is the wavelength of X-ray used (1.5405 A), β is the angular peak width at half maximum in radians and θ is the Bragg's diffraction angle. The average particle sizes are calculated to be around 25 nm. The observed diffraction peaks were broad around their bases, signifying that the ZnO nanoparticles are in a nanosized regime (Kulkarni and Shirsat, 2015; Xaba*et al.*, 2016).

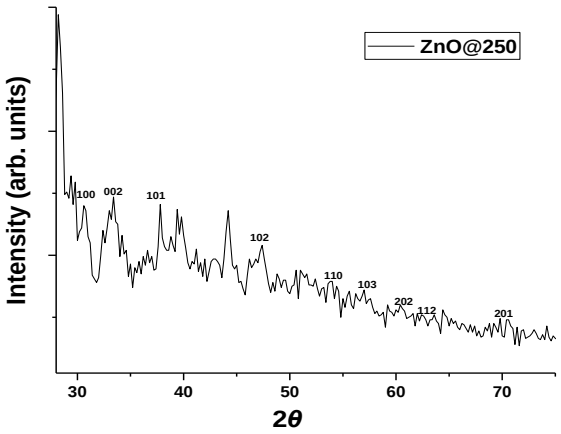


Figure 5: XRD diffraction pattern of ZnO nanoparticles

Transmission Electron Microscopy (TEM) Studies

The morphological characteristics of the zinc oxide nanoparticles revealed by TEM images are shown in Figure 6. The TEM images of ZnO NPs demonstrate the presence of nanocrystalline particles and aggregation with different shapes and sizes depending on the temperature rate used for nanoparticle synthesis (Kalpanadevi*et al.*, 2013; Xaba*et al.*, 2016; Gharibshahi*et al.*, 2017). ZnO NPs were prepared by decomposition method, possess slightly spherical, and are loosely distributed with an average particle size in the range of 23 nm and 30 nm. The average particle size of ZnO nanoparticles obtained from TEM image analysis is consistent with the average crystallite size as assessed by XRD analysis.

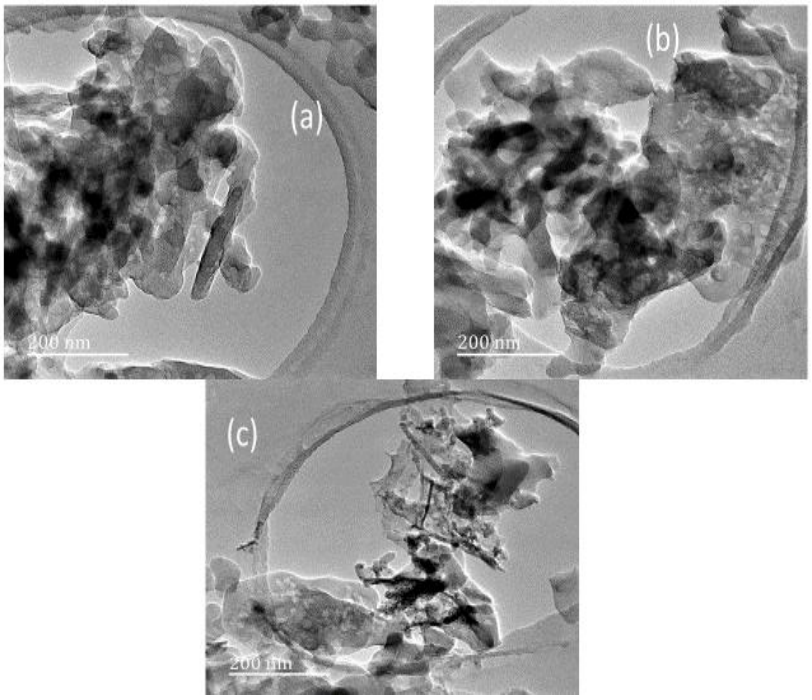


Figure 6: TEM micrographs of ZnO NPs: (a) ZnO@250, (b) ZnO@300, (c) ZnO@350

Biological evaluation of ZnO nanoparticle

The antioxidant activity of the ZnO nanoparticles was studied spectrophotometrically using the DPPH method alongside the standards agents- gallic acid and ascorbic acid, as displayed in Figure 7. (Ejidike and Ajibade, 2017). Radical scavenging activity of nanoparticles, as well as the standards, exhibited appreciable activities. However, the activities of standard drugs (gallic acid and ascorbic acid) possess higher antioxidant potential (IC_{50}) than ZnO nanoparticles. The DPPH radical scavenging activity decreased as follows: Ascorbic acid > Gallic acid > ZnO@350 > ZnO@250 > ZnO@300. The scavenging potentials of the ZnO nanoparticles could be ascribed to electron density transfer located at oxygen to the odd electron located at nitrogen atom in DPPH (Stan *et al.*, 2016). The scavenging potentials of the nanoparticles are associated with the presence of negatively charged active compounds such as COO^- and O^- of the ligand and positively charged nanoparticles ($ZnO = Zn^{2+} + O^{2-}$) that exerted electrostatic attraction required for the breaking of free radical chains (Kumar *et al.*, 2014; Stan *et al.*, 2016; Ejidike, 2018). The differences in the scavenging activity of the synthesized ZnO using the ONNO Schiff base zinc complex could be related to the fact that the nanoparticles possess different sizes and specific surfaces according to the different temperatures used in the synthesis process. The ZnO@350 samples which have the smallest nanoparticle size showed the highest DPPH scavenging activity amongst the nanoparticles.

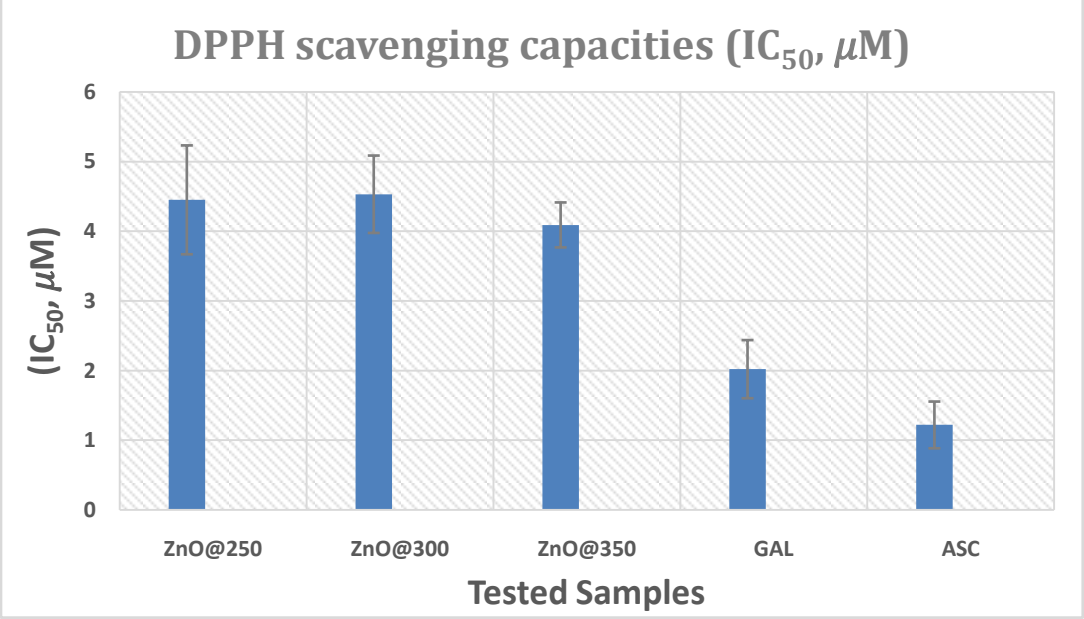


Figure 7: DPPH scavenging capacities (IC_{50}, μM); ASC = Ascorbic acid and GAL = Gallic acid

Conclusion

The main goal of the present work is to synthesize Zinc Oxide nanoparticles from the Schiff base complex and their application as antiradical agents. Zinc ion to ligand binding was confirmed by spectral analyses. Ligand-Zn complex was observed to possess tetrahedral geometry. The thermal stability of the complexes was obtained by TGA studies. Formation of the ZnO nanoparticles synthesized from Zn(II) complexes were confirmed by FT-IR, UV-Vis, XRD, and TEM spectra. The energy band gap of the synthesized nanoparticles is approximately 3.15 eV for ZnO@250, 3.31 eV for ZnO@300, while ZnO@350 was found as 3.17 eV and 3.56 eV. The

nanoparticles were also evaluated for their free radical scavenging property and observed varying antioxidant activities as compared to standard drugs showed higher DPPH scavenging potentials than the various ZnO nanoparticles. The results from DPPH methods revealed that the compounds are capable of donating an electron, consequently, then react with free radicals or terminate chain reactions in a dose-dependent pattern.

Acknowledgment
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ORIGINAL RESEARCH ARTICLE

The enhanced photovoltaic performance of perovskite solar cell using carbon nanotubes as hole transport material

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ABSTRACT

Perovskite based solar cells have enjoyed rapid and an unprecedented evolution over the past decade. These light-harvesting materials are of huge interest to the academic community in order to make more efficient solar cells which are expected to attain swift commercialization. They have attractive properties, most especially their high-power conversion efficiency (PCE) within few years in comparison to other third generation thin film technologies. In This research work, the effect of incorporating multi-walled carbon nanotubes as the hole-transport layer on the photovoltaic performance of perovskite solar cells was investigated. UV-Vis spectrophotometry, Scanning Electron Microscopy, Surface Profilometer, Raman Spectroscopy and Solar Simulator were used to characterize and study the properties of the prepared cells. The reference cell demonstrated a PCE, current density (Jsc), open circuit voltage (Voc) and fill factor (FF) of 2.82 %, 7.64 $mAcm^{-2}$, 0.88 V, and 42.00 % respectively. When multi walled carbon nanotubes (MWCNTs) was incorporated, we observed a PCE of 4.30 %, Jsc of 8.47 $mAcm^{-2}$, Voc of 0.85 V and FF of 60.00 %. The MWCNTs modified device shows an enhancement of 52.48 % in PCE, 10.86 % in Jsc, and 42.86 % in FF over the unmodified device. This is due to improved surface area of MWCNTs by acid treatment in generating functional groups that act as conducting bridge in reducing the contact resistance between individual nanotubes.

Key words: *Perovskite, power conversion efficiency, multi-walled carbon nanotubes, hole transport material*

INTRODUCTION

Natural sources of energy such as sunlight, rain, wind, tides, geothermal heat and waves are replenished in abundance. They offer great opportunities to be utilized as renewable energy sources in meeting up with the increasing energy needs around the world. Renewable energy also can be employed as suitable alternatives to fossil fuels and other sources of energy. By utilizing and deploying modern technology of renewable energy sources, there would be economical growth as well as global energy security. This will also reduce environmental pollution caused by burning of fossil fuels and further improve public health.

The world demand for energy is rapidly growing. For example, in 2016, the total worldwide energy consumption was approximately 1.33×10^8 tonnes of oil equivalent (toe) (BP Statistical Review of World Energy 2017), which corresponds to roughly 1.5×10^5 terawatt hours (TWh). These numbers rise continuously as the world population and economy grows. Therefore, to invest in clean and environmentally friendly energy sources such as solar energy is the way forward. Every year the sun provides the Earth's surface with 1.9×10^8TWh of radiation (Golusin, Dodic, and Popov, 2013); this implies that it supplies in about 7 hours enough energy needs for one year. Furthermore, solar energy is a clean, readily available, free and unlimited. A great challenge, however, is the large gap that exist between current utilization of solar energy and its untapped potential.

Globally, there has been an increase in the quest to embrace alternative sources of energy such as renewables, because of the negative environmental impact of burning fossil fuels and their gradual exhaustion. Solar energy is the dominant energy source in the world. In order to convert solar energy into electric energy that can be directly used by humanity, different kinds of solar cells have been developed, among which is the photovoltaic (PV) cells. Photovoltaic (PV) cells are devices that convert sunlight into electrical power and have great potential to meet society's continuously growing energy demands with negligible negative environmental impact. Solar cells technologies are grouped into three generations, namely: first, second and third generation solar cells (Green, 2001).

The general chemical formula for halide perovskite materials is given by ABX_3. Where X is either oxygen or halogen (anion), A and B are cations, A is larger than B. The A cation occupies a cubo-octahedral site shared with twelve X anions, while the B cation is stabilized in an octahedral site shared with six X anions (Figure 1) (Park, 2015).

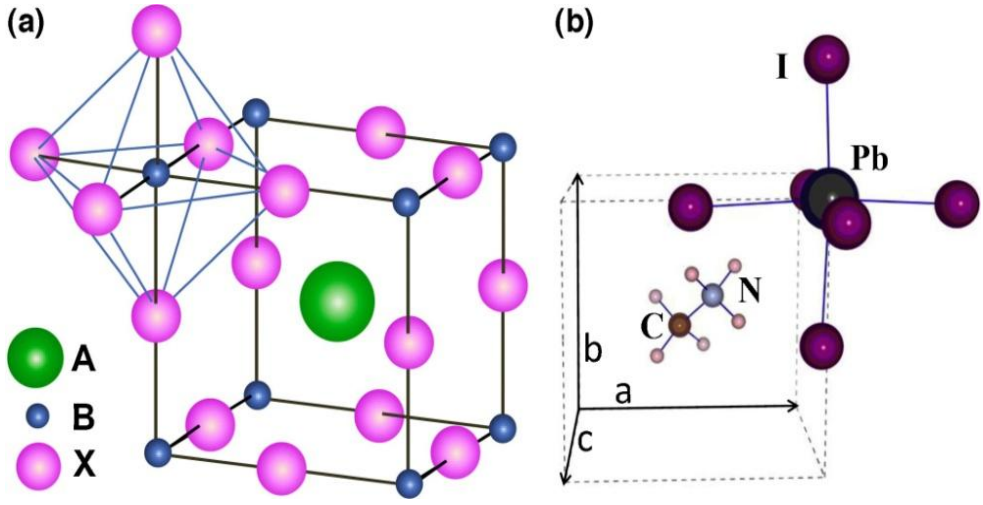

Figure 1: (a) ABX_3 perovskite structure showing the BX_6 octahedral and larger A cation occupied in the cubo-octahedral site; (b) unit cell of cubic $CH_3NH_3PbI_3$ perovskite. (Park, 2015)

In the perovskite chemical formula, A is typically a small organic cation like $CH_3NH_3^+$ (methyl ammonium), $C_2H_5NH_3^+$ (ethyl ammonium) or $HC(NH_2)_2^+$ (formamidinium). The charge of

Cation A is +1, and it is the most vital component of the perovskite molecule, because it determines the structure and the size of perovskite crystal and has direct influence on the stability and optoelectronic properties of perovskite material (Boix*et al.,* 2015). B usually is made up of metal ion with a charge of +2 like Pb^{2+}, Sn^{2+} or Cu^{2+}, and X is usually Cl^{-}, Br^{-} or I^{-}.

Several progresses have been made in perovskite solar cell (PVSC) research, but a number of key issues needs to be addressed before commercialization can be affected. A major challenge is the long-term stability of the $CH_3NH_3PbI_3$ material in the presence of water, UV irradiation, O_2 polarization and ion migration. Moisture significantly degrades $CH_3NH_3PbI_3$ into PbI_2 and CH_3NH_3I (MAI). The degradation reaction comes into play when a molecule of water interacts with the proton present in the $CH_3NH_3PbI_3$ crystals and ultimately degrades it to form aqueous HI, solid PbI_2, and volatile CH_3NH_2 (Aristidou*et al*., 2015 and O' Mahony *et al*., 2015). In the case of oxygen, it is thought that a superoxide is generated through an electron transfer from the photoexcited $CH_3NH_3PbI_3$ to an O_2 molecule. The superoxide that is formed, tends to attack the $CH_3NH_3PbI_3$ absorber chemically making it to decompose into CH_3NH_2, PbI_3, I_2 and water (Aristidou*et al*., 2015). A key to protect these $CH_3NH_3PbI_3$ perovskites from moisture, UV and O_2 is by using a very effective protective coating. The perovskite solar cells (PVSCs) stability may be enhanced by optimizing different parts of the device and its constituents such as the perovskite crystal structure, cell encapsulation, quality of film, alternative designs, conducting layers and interfaces (Yang and You, 2017). In a bid to address these challenges, Habisreutinger*et al*. (2014) deployed an intelligent technique. They coated perovskite films with poly 3 hexylthiophene/single walled carbon nanotubes (P_3HT/SWCNTs) then with poly methyl methacrylate (PMMA). The P_3HT/SWCNTs-PMMA composite structure forms a good conducting material as well as protecting the perovskite layer from degradation. The experimental device architecture for the cell with the best stability was FTO/TiO_2/Al_2O_3+$CH_3NH_3Pbl_3$/P_3HT-SWCNTs/PMMA/Ag. Aitola*et al*. (2017) investigated high temperature stability properties of SWCNT-Spiro-OMeTAD and compared with gold (Au) electrode-based devices in another experiment. The device architecture with an enhanced performance in the experiment was FTO/c-TiO_2/m-TiO_2/$CH_3NH_3Pbl_3$/SWCNT-Spiro-OMeTAD. The carbon nanotubes (CNTs) do not only possess the ability to transport holes, but also possess the tendency to repel moisture (hydrophobic property) that is capable of degrading PVSCs.
This research project basically aims at investigating the photovoltaic performance of multiwalled carbon nanotubes incorporated at the hole-transport layer in mesoscopic perovskite solar cell. In this study, carbon-based electrode will be deployed because carbon materials are readily available, less expensive and anti-corrosive.

MATERIALS AND METHODS

The Main Materials

Titanium Nanoxide TSP-36, Titanium Isopropoxide, Zirconium Nanoxide, Methyl AmmoniumIodide (MAI), Lead (II) Iodide, N, N Dimethyl Formamide (DMF), Acetyl Acetone, Fluorine doped Tin Oxide (FTO), Potassium Per Manganate ($KMnO_4$), Multi-Walled Carbon Nanotubes (MWCNTs), 2-Propanol, Glass slides, Methanol/Ethanol, Concentrated Hydrochloric Acid (HCl), Concentrated Tetraoxosulphate (vi) Acid (H_2SO_4), Sodium Hydroxide (NaOH), Sodium Laureth Sulphate, Polyvinyl Alcohol (PVA), Hydrogen Peroxide (H_2O_2), Nitrogen gas, Aluminium foil, Elco-carbon, Distilled /deionized water.

The Main Equipment

Whirling Hygrometer, Sonicator, Electrified Engraver (SWISCO 220/250 V_{AC}, glass cutter),Magnetic Hot Plate Stirrer 78-1, Spin-Coating Centrifuge, Electronic Weighing Balance, Glove Box, Hot Air Blower, Beakers, Filter Papers, Test tubes, Scanning Electron Microscope (SEM), Raman Spectroscopy, Infra-Red Thermometer, UV Spectrophotometry, Profilometer, 4-Point Probe, Temperature Regulator, Solar Simulator.

Preparation of Films and Precursors

Synthesis of Carbon Nanotubes (CNTs)

MWCNTs were synthesized at Federal University of Technology Minna, using the Chemical Vapour Deposition Method (Schultzenberger and Schultzenberger, 1890). The procedure involves passing a hydrocarbon gas for 45 minutes through a tubular reactor in which an iron catalyst material is present at a very high temperature 900 $^{\circ}$C to decompose the hydrocarbon. The CNTs grow on the metallic catalyst that is present in the reactor, which are collected when the system cools at room temperature (Mukul and Yoshinori, 2010). CNT formation is guided by the size of the catalyst particle. Particles with a few tens' nm wide favours the formation of MWCNTs (Sinnott *et al*., 1999).

Purification of MWCNTs

In purifying the MWCNT, 1g of MWCNT was measured and put in a beaker. 50ml of concentrated HCl was added to the MWCNT in a beaker. The mixture was stirred and heated simultaneously using a magnetic stirrer hot plate (model 78-1) where a temperature of 80 $^{\circ}$C was maintained for 4 hours. Thereafter, the mixture was filtered using a filter paper. The residue is then rinsed with methanol at room temperature. This was followed by further heating of the mixture to dry at a temperature maintained at 100 $^{\circ}$C for 1 hour. 0.1g of the cleaned MWCNT was measured and added to 10 ml of N, N Dimethyl Formamide (DMF). The mixture was sonicated using a Sonicator (100 W, 40 kHz) for 30 minutes so as to deagglomerate the MWCNTs.

Preparation of Compact Titanium dioxide (c-TiO_2) Precursor

The c-TiO_2 is a nanoporous metal oxide that reduces recombination. The compacted layer thickness is optimized to offer minimum resistance to drift velocity of photo-generated electron. Two different concentrations were used:

The first c-TiO_2 was low in concentration: 0.3M of Titanium Isopropoxide + 1.2M Acetyl Acetonate + 0.15M Propanol.

The second c-TiO_2 was high in concentration: 0.3M of Titanium Isopropoxide + 1.2M Acetyl Acetonate.

Preparation of Mesoporous Titanium dioxide (m-TiO_2) Film

Commercially available Titanium Nanoxide T/SP 36 paste purchased from solaronix was used. The Titanium Nanoxide T/SP 36 was diluted in absolute ethanol in the ratio 1:3 respectively to obtain the required composition for preparing the m-TiO_2 film. The prepared m-TiO_2 solution was sealed with an aluminium foil to prevent it from absorbing moisture.

Preparation of Dried Isopropanol

In a bid to get rid of the absorbed moisture in Isopropanol, 600 ml of Isopropanol was mixed with 60 g of NaOH. The mixture was allowed to undergo a distillation process. Thereafter, the dried Isopropanol was collected and stored in a well-sealed container to prevent it from moisture.

Preparation of Methyl Ammonium Lead Triiodide ($MAPbI_3$) Precursor

Lead (II) Iodide (PbI_2) and Methyl Ammonium Iodide (MAI) solution was prepared separately. Firstly, in a bid to dissolve PbI_2; 4.61 g of PbI_2 was mixed with 10 ml of DMF in a test tube and heated at 250 $^{\circ}$C inside a molten wax bath which serves as a heat transfer medium. This temperature was maintained until PbI_2 dissolves. The solution was stirred to prevent the crystallization of PbI_2. Face mask was used as a safety precaution to prevent the harmful effect of lead.

Secondly, 0.32 g of MAI was mixed with 20 ml of propanol stirred manually for 3 minutes.MAI is prepared just before its use because it is highly sensitive to moisture.

Preparation of the FTO glass slides

Firstly, the FTO glass was cleaned using cotton wool to get rid of dirt. Electrified engraver (SWISCO 220/250 $V_{AC)}$ was used to cut the FTO glass in square shape (2.5cm by 2.5cm).

The FTO glass slides were put in a beaker and then washed with 5 % Sodium Laureth Sulphate (SLS) and 100 ml of water. In order to get rid of the excess foam, deionized water was used to rinse the glass slides in the beaker. After rinsing, the glass slides were dried using a magnetic stirrer hot plate 78-1. The temperature of the hot plate was measured using infra-red thermometer. The glass slides were later sonicated for one minute using a Sonicator so as to remove impurity.

Preparation of MWCNTs Film

0.008 g of Polyvinyl Alcohol (PVA) was added to 0.006 g of MWCNTs, 0.4 g of Sodium Laurent Solution (SLS). The mixture was sonicated for 5 minutes. Spray deposition technique was employed in depositing the MWCNTs on the glass slide and dried at 80 $^{\circ}$C. The glass/MWCNTs was rinsed with acetone so as to remove the SLS. Glass/MWCNTs was further rinsed in deionized water then annealed at 400 $^{\circ}$C.

Device Fabrication

Fabrication of Photo-Anode

We begin the fabrication process of the photo-Anode by depositing the c-TiO_2 on the already cleaned FTO glass. The FTO glass is gently removed from the beaker where it was stored under deionized water using forceps and placed on a hot plate where it was heated to dry up the water content. When water was no longer present on the FTO glass, the conductive side of the FTO was identified using a multi meter and 10 drops of the already prepared c-TiO_2 solution were dropped on the FTO and spin-coated for 20 seconds at 3000 rpm (see figure 2a). For the film to properly form, the FTO/c-TiO_2 was annealed at 400 $^{\circ}$C for 30 minutes. This was followed by dropping m-TiO_2 solution on top of the c-TiO_2 layer and spin-coated for 20 seconds at 3000 rpm. The m-TiO_2 layer was dried thermally at 80 $^{\circ}$C using a hot plate for 10 minutes (see figure 2b). The sintering of the glass/FTO/c-TiO_2/m-TiO_2 layer was done at 160 $^{\circ}$C.

Sequential Deposition of Perovskite ($MAPbI_3$) Layer

In this work Methyl Ammonium Lead Iodide perovskite, $CH_3NH_3PbI_3$ is formed using sequential deposition method in which perovskite is solution-processed in two steps as shown in figure 2c. First PbI_2 is spin-coated on top of glass/FTO/c-TiO_2/m-TiO_2 layer at 3000 rpm for 10 seconds. The PbI_2 film is dried at 70 °C on hot plate for 10 minutes. Then the substrates are immersed vertically in the MAI solution for 10 seconds. Perovskite is formed in the solution as a result of the reaction between PbI_2 and MAI. After the immersion, the films were rinsed with dried propanol to remove the unreacted MAI from the surface of perovskite.

The Perovskite was formed in air with relative humidity of 65 %. The ambient temperature during the fabrication was 26 °C.

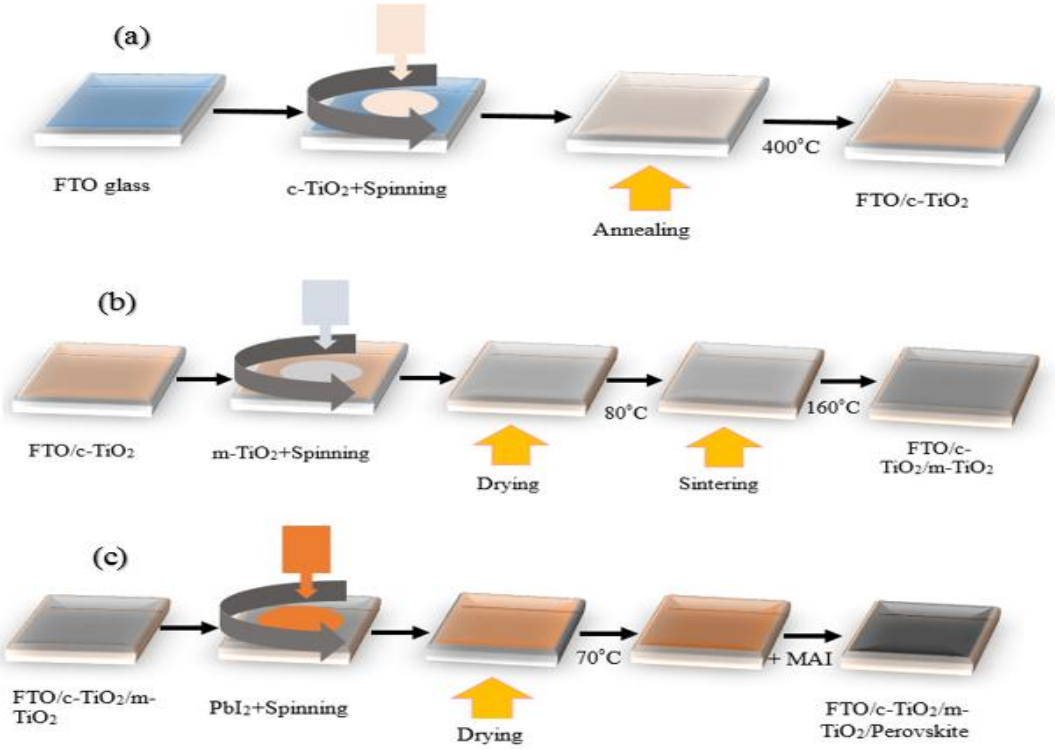

Figure 2: **Schematics for the deposition of (a) compact titanium dioxide on FTO glass, (b) mesoporous titanium dioxide on FTO glass/c-TiO_2 film and (c) perovskite on FTO/c-TiO_2/m-TiO_2 film.**

Fabrication of Counter Electrode

The counter electrode used was Elcocarb. Elcocarb is a graphite/carbon black paste produced by solaronix.

Fabrication of the Cell

In line with the objectives of this research study, two cells were fabricated. The first cell is the reference cell without MWCNT. The second cell is incorporated with MWCNT on top of the perovskite layer.

First Cell

This is the reference cell that forms the basis for comparison with the cell incorporated with MWCNT at the HTL. The counter electrode (Elcocarb) was laminated on the photoanode at a temperature of 150°C for 5 minutes to obtain the Glass/FTO/c-TiO_2/m-TiO_2/ZrO_2/$MAPbI_3$/Elcocarb cell architecture.

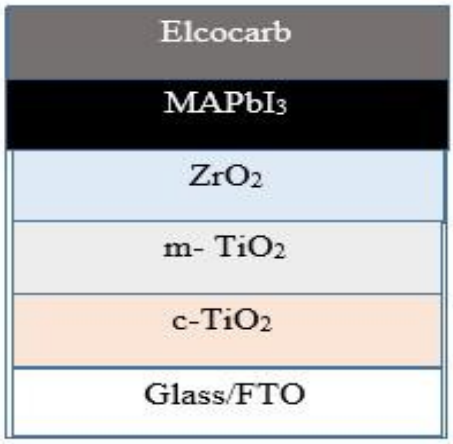

Figure 3: First cell architecture

Second Cell

This is the cell incorporated with MWCNT at the HTL. The counter electrode (Elcocarb) was laminated on the photoanode at a temperature of 150 °C for 5 minutes to obtain the Glass/FTO/c-TiO_2/m-TiO_2/ZrO_2/$MAPbI_3$/MWCNT/Elcocarb cell architecture.

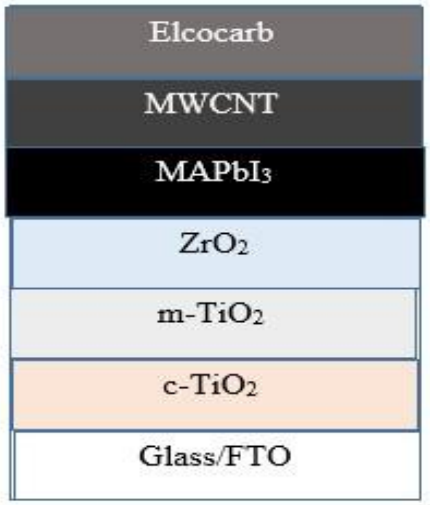

Figure 4: Second cell architecture

Characterization and Measurement

In the course of this study, different instruments were used for various characterization and measurement to achieve the desired objectives.

Ultraviolet-Visible Spectrophotometer was used to study the optical properties such as intensity and wavelength of absorption of the formed photo anode films.

Scanning Electron Microscopy (SEM) operating at an accelerated voltage of 15 kV was used to study the surface morphologies of the formed films.

Surface Profilometer for determination of film thickness.

Raman Spectroscopy a viable tool for the characterization of carbon-based nanostructures was used to study the quality of the MWCNT.

The current-voltage (I-V) measurement of the formed cells was carried out under AM 1.5G ($100mWcm^{-2}$) solar illumination.

RESULTS AND DISCUSSION

SEM Result Analysis for m-TiO_2

The structural morphology of the m-TiO_2 was also studied using Scanning Electron Microscopy, SEM (Phenom Pro PW-100-012 model) operating at an accelerated voltage of 15 kV. The image presents a homogenous film with uniform thickness and morphology as shown in Figure 5.

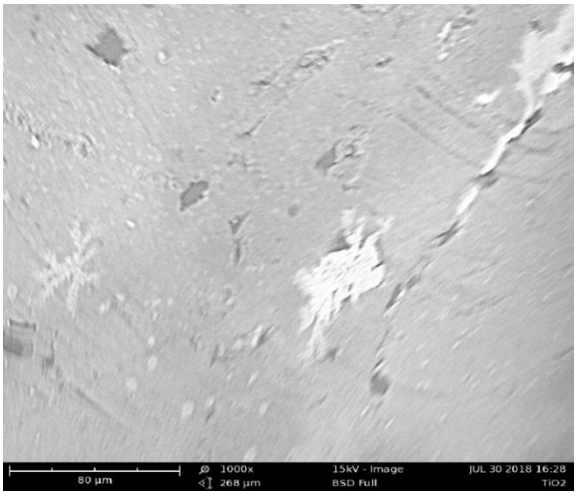

Figure 5: SEM image for m-TiO_2.

The surface is dense and presents the crystalline nature of the produced film annealed at 400 °C. The crystalline nature of anatase TiO_2 may be due to several factors such as: the large surface area of TiO_2 creating dangling bonds and creates electronic states through the energy gap acting as trapping levels (Mott and Allgaier, 1967; Barzykin and Tachiya, 2002) which is responsible for the crystalline nature of TiO_2 and its expansive background. The strength of the bond, the average coordination number, and heat capacity could produce the sustained thermal stability and the weak response to annealing of anatase TiO_2 at 160 °C. In addition to the factors mentioned, the deposition of the substrate at room temperature, and localized deformation, method of preparation, type of substrate, heteropolar and homopolar bonds energies can affect the structural stability and the response of TiO_2 to external changes. The surface morphology

also presents particle agglomeration. The image also shows the presence of several macroscopic defects which might result from the handling of the sample during fabrication and characterization.

SEM Result Analysis for MWCNT

Analysis of morphological surface of carbon nanotube was performed by Scanning Electron Microscopy, SEM (Phenom Pro PW-100-012 model) operating at an accelerated voltage of 15 kV. The image is as shown in Figure 6

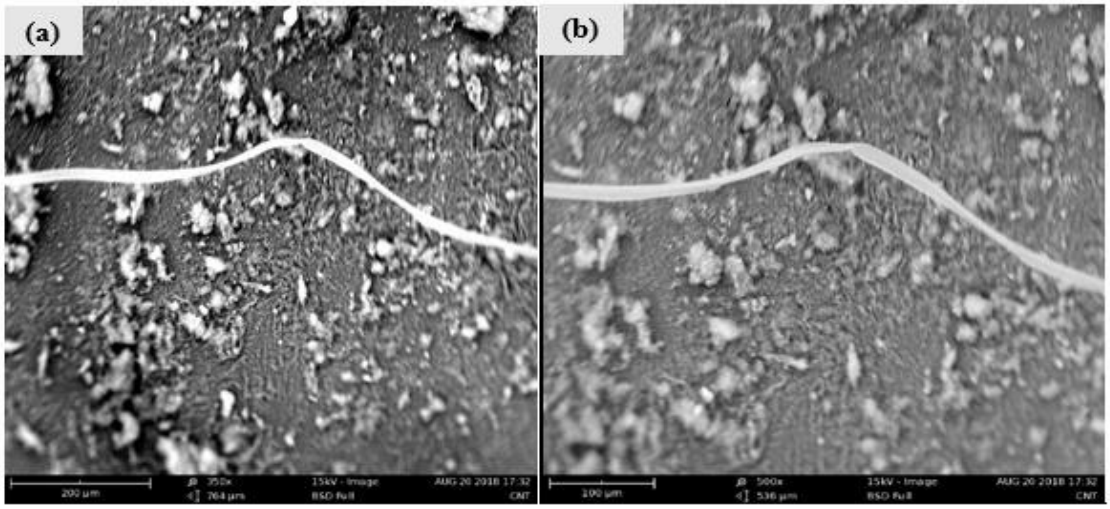

Figure 6: SEM images for MWCNT

Figures 6a and b present the SEM image for MWCNT. The image presents deagglomeration of MWCNTs particles that are well-dispersed, after thermal and acid treatment in order to get rid of impurities such as graphite and metal catalyst. The acid treatment is necessary so as to intercalate and exfoliate graphite and other impurity materials thereby creating large numbers of oxidation sites on the carbon atom (Liu *et al*., 1998, Chen *et al*., 2001 and Zhang *et al*., 2003).
The SEM image indicates that the nanotube is visible after the acid treatment.

Surface Profilometer for Perovskite, m-TiO_2 and purified MWCNT

The profiling results for perovskite, m-TiO_2 and purified MWCNT are presented in Figures 7(a), (b) and (c) respectively

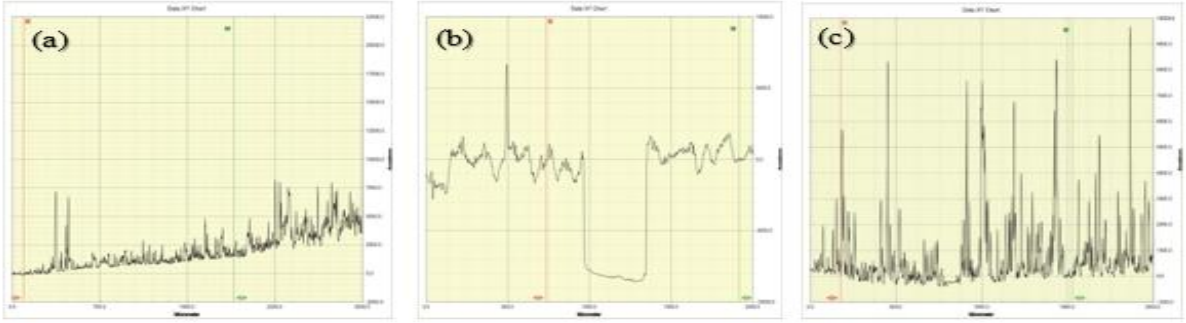

Figure 7:Surface profiling for (a) perovskite (b) m-TiO_2 and (c) purified MWCNT

The film thicknesses were obtained with a surface profiler.

UV-Vis Spectrophotometry Result Analysis

The absorption spectra were obtained for m-TiO_2, perovskite and m-TiO_2/perovskite films using ultraviolet–visible spectrophotometer and presented below:

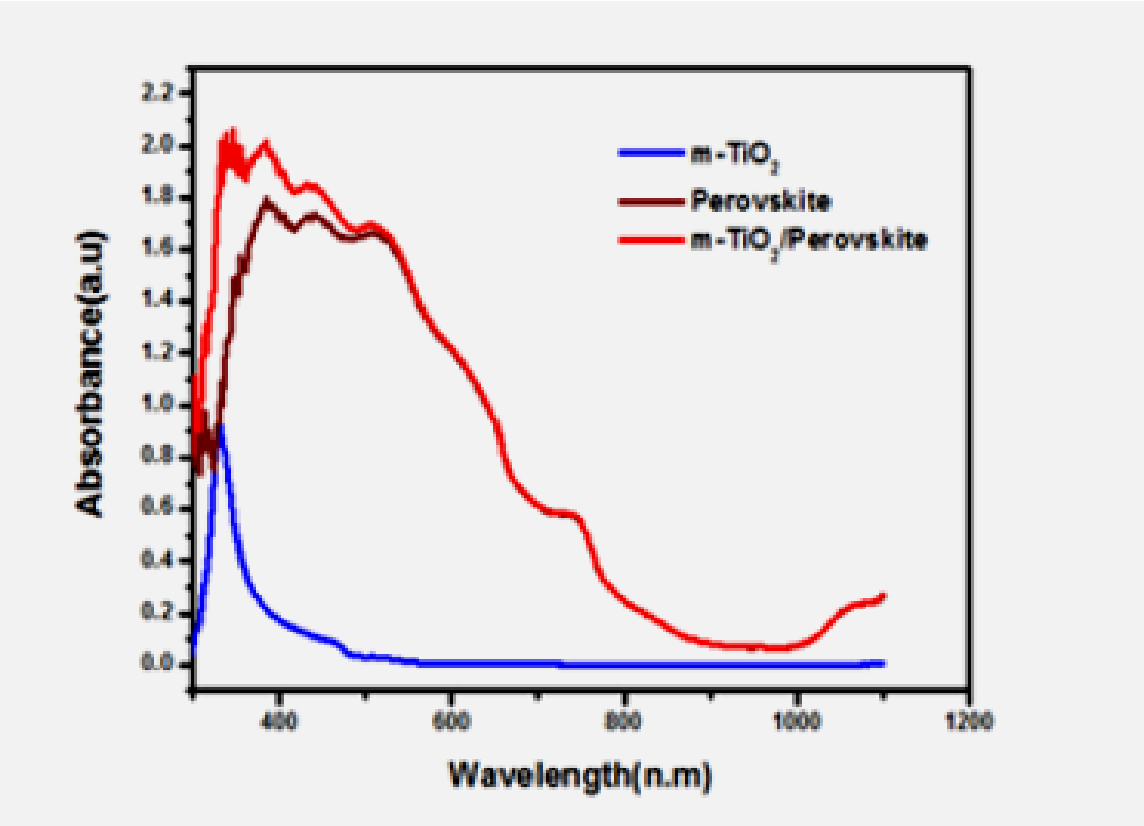

Figure 8: UV-Vis spectra for m-TiO_2, perovskite, and m-TiO_2/perovskite thin film

From the graph of absorbance against wavelength for pure m-TiO_2 in Figure 8, an absorbance peak of wavelength 332 nm was observed. This absorbance peak is due to electronic transition between the molecule having an intermediate ionic degree in conformity with those of the synthesized molecular material. At such a wavelength, no visible light can be absorbed since m-TiO_2 is absorbing within the ultra-violet region. The graph shows that m-TiO_2 was not capable of harvesting visible light within the solar spectrum. The inability of m-TiO_2 to absorb light therefore, indicates that it will not inhibit the transmittance of light to the absorbing layer of the about-to-be-formed solar cell. The m-TiO_2 here is acting as an insulator because between the visible and near infrared region there is no obvious absorption peak present. Hence, m-TiO_2 has not met the requirement to be used as a light harvester. Therefore, the activation of m-TiO_2 with a modified nano material will shift the energy level to a negative potential in a quasi- state and make it absorb photons at longer wavelength up to near infrared region of the solar spectrum. This will narrow the energy gap and increase conductivity.

The brown coloured spectra in figure 8 is the graph of absorbance against wavelength for perovskite film. The need for absorbance peak and wavelength is to find out if the perovskite material has met the requirement to be used as a light harvester in the PVSCs that is about to be formed. The graph reveals four absorbance peaks corresponding to absorbance wavelength of 392 nm, 444 nm, 519 nm and 750 nm. This shows that perovskite absorbs light within the visible region with longer wavelength range of between 390-800 nm. The graph shows that the perovskite material has a high absorption coefficient within the visible region of the solar spectrum. Hence, it has satisfied the major requirement to be used as a light harvester in this research. Therefore, modifying the m-TiO_2 with a perovskite material is a major necessity to achieving the stated objectives.

The red coloured spectra in figure 8 indicates that the activation of m-TiO_2 by the perovskite material results in a red shift (bathochromic effect) which is a positive shift of the absorbance wavelength towards the red region of the solar spectrum, leading to longer absorbance wavelength and the broadening of the absorption spectra due to a change in the band gap of the material. The presence of auxochrome such as methyl and halogen substituents in perovskite, enhances the shifting of the absorption maximum to longer wavelength (Subodh, 2006). The implication of this is the decrease in energy difference between the highest occupied molecular orbital (HOMO) and lowest unoccupied molecular orbital (LUMO) of the resulting m-TiO_2/perovskite layer. This change in energy molecular orbital (MO) on modification results in absorption of radiation at longer wavelength. The modification not only results in bathochromic shift (longer wavelength) but also increases the intensity of absorption (hyperchromic effect) due to the increase in the number of delocalized electrons leading to a quasi-fermi level. The brown and red spectra in figure 8 shows no significant change in the absorbance wavelength. However, there is an increase in the intensity of absorption as evident by higher absorbance peaks for the m-TiO_2/perovskite film. This high absorbance intensity is attributed to the presence of simple un-conjugated chromophore with lone pair electrons in the m-TiO_2/perovskite film. As a result, there is a high energy transition from an occupied molecular orbital (a non-bonding π orbital) to an unoccupied molecular orbital (σ* orbital) of greater potential energy. i.e., $\pi \rightarrow \sigma^*$ (Subodh, 2006). The broadening of the wavelength and the intensity of the absorption is indicative that the material used is efficiently acting as an antenna in harvesting and transporting photo excited species.

The Band Gap Measurement (E_g)

The band gap of m-TiO_2, perovskite and m-TiO_2/perovskite film is calculated by Tauc's plot (Kim and Jeong, 2007) which is expressed as:

$(\alpha h\upsilon)^{\frac{1}{n}} = (h\upsilon - E_g)$, $\alpha = \frac{\text{Absorbance}}{\text{Thickness}}$ where α is the coefficient of absorption, h is Planck's constant, υ is frequency (υ=c/λ, c is the speed of light, λ is the wavelength), n=1/2 for direct optical band gap, and E_g is band gap which is estimated by extrapolation of the linear portion to intercept the abscissa.

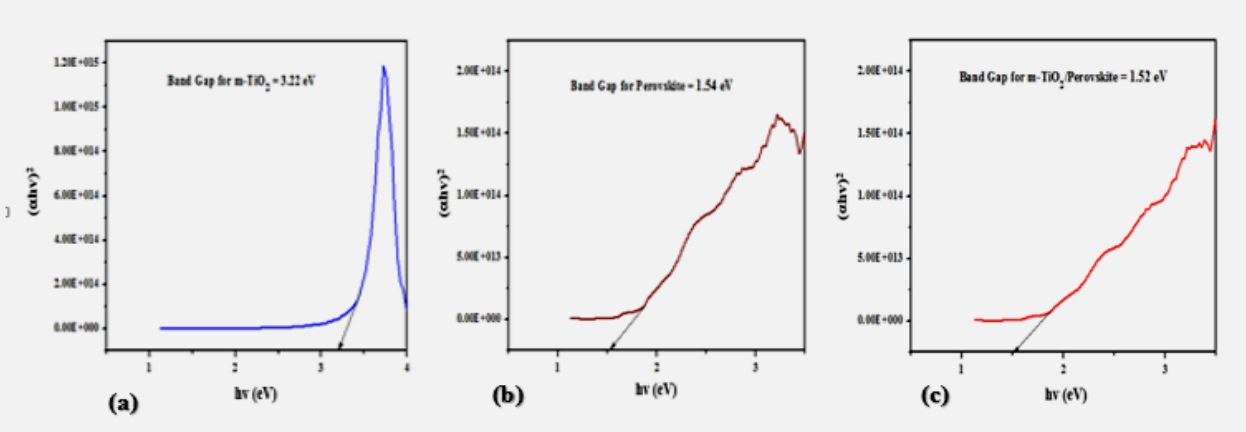

Figure 9: Tauc's plot showing the Band Gap for (a) m-TiO_2, (b) perovskite and (c) m-TiO_2/perovskite film

The band gap of the synthesized m-TiO_2, perovskite and m-TiO_2/perovskite is 3.38, 1.54 and 1.52 eV. The band gap value of 3.38 eV is close to the already reported value of 3.2 eV for m-TiO_2, while the band gap value for perovskite is within the acceptable range of 1.5-1.6 eV.

MWCNT Raman Spectroscopy Result Analysis

The quality of the MWCNT was carried out using Raman spectroscopy.

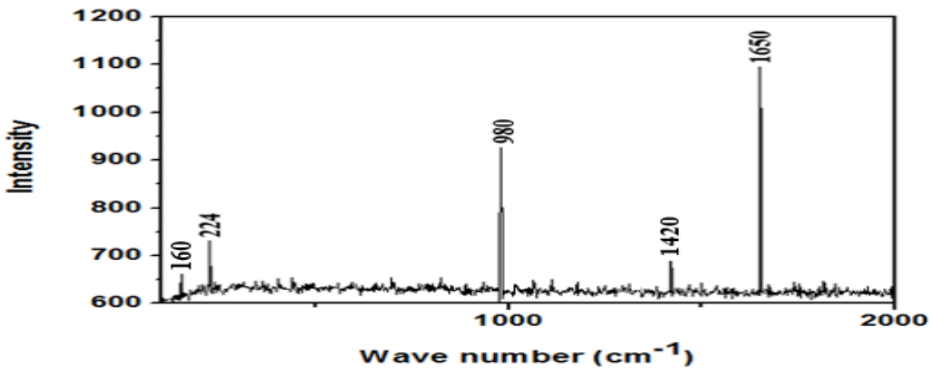

Figure 10: Raman spectra of purified MWCNT

As shown in Figure 10 above, the Raman spectra presents major peaks at 160 cm^{-1}, 224 cm^{-1}, 1420 cm^{-1} and 1650cm^{-1}. Two peaks of radial breathing modes (RBM) were observed to be in the range of 160 cm^{-1}-224 cm^{-1}, suggesting that semiconducting MWCNTs with diameters of 1.1-1.6 nm were excited resonantly through the interband transitions of E_{22}^{S} (Kataura, *et al*., 1999). The observed peak at 1420 cm^{-1} correspond to the disorder-induced modes (D-band). A very high peak observed at 1650 cm^{-1} correspond to the tangential graphite-like modes (G-band), also suggest that the semiconducting MWCNTs were in resonance (Kim *et al*., 2005). This is also indicative that carbon nanotubes have been synthesized from high quality graphite. The peak at 980 cm^{-1} could be as a result of silicon oxide or hydroxide probably from the sample holder or

the template which was used during characterization. The Raman spectra shows that the acid-treatments did not alter the structural ordering of MWCNTs.

Characterization and Evaluation of the formed cells

The solar simulation was carried out to determine the optoelectronic properties of the fabricated cells. The current-voltage (J-V) measurement was performed to obtain the efficiency of the PVSCs. Important factors that characterize solar cells are the short circuit current density (Jsc) and the open circuit voltage (Voc). Jsc is the current of the cell without applied bias voltage. It provides insights about the absorption behaviour, the production of charge carrier and the charge carrier movement within the absorber layer. Voc is the required voltage that compensates the internal electrical field of the solar cell. This means that no current is flowing at V_{OC}.

The power conversion efficiency (PCE) is directly proportional to the ratio of maximum power (Pmax)and the power of the incident light (Pin). The fill factor (FF) is introduced as another quality factor, which varies as a function of the shunt and series resistance of the device. The values for the FF and PCE were determined from the following equations:

$$FF\ (\%) = \frac{V_{max} \cdot J_{max}}{V_{oc} \cdot J_{sc}}, \quad PCE\ (\%) = \frac{FF \cdot V_{oc} \cdot J_{sc}}{P_{in}}$$

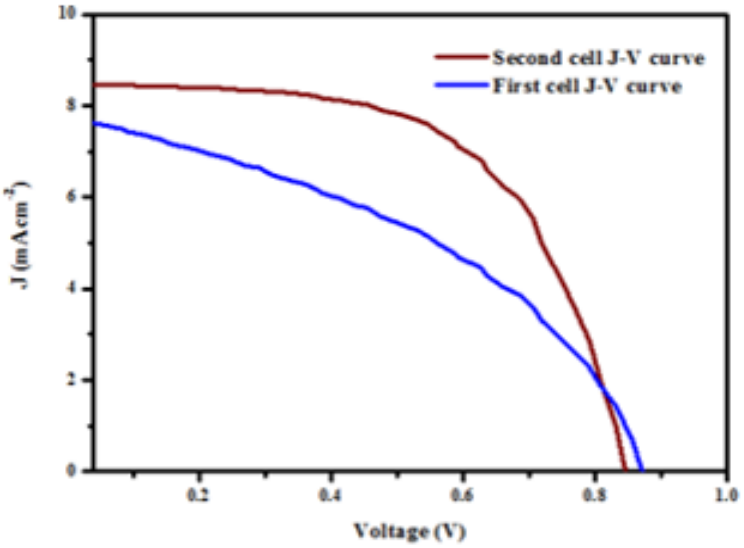

Figure 11: J-V curve for the first and second solar cells

Table 1: The photovoltaic parameters for the first and second solar cells

Device	Architecture	J_{SC} ($mAcm^{-2}$)	V_{OC} (V)	FF (%)	PCE (%)
1	Glass/FTO/c-TiO_2/m-TiO_2/ZrO_2/$MAPbI_3$/Elcocarb	7.64	0.88	42.00	2.82
2	Glass/FTO/cTiO_2/mTiO_2/ZrO_2/$MAPbI_3$/MWCNT/Elcocarb	8.47	0.85	60.00	4.30

Current density-voltage (J-V) curves was obtained after exposing the formed solar cells to AM 1.5G (100 $mWcm^{-2}$) solar illumination in order to test their photovoltaics performance. The photovoltaic performance parameters (Jsc, Voc, FF and PCE) is summarized in Table 1 above. The incorporation of MWCNTs into the second device produced an increase in the value of Jsc from 7.64 $mAcm^{-2}$ to 8.47 $mAcm^{-2}$ with an enhancement value of 10.86%. This is due to enhanced charge extraction and transfer ability induced in the second device by the introduction of MWCNTs. The FF increased from 0.42 to 0.60 with an enhancement value of 42.86%. This indicates that the incorporation of MWCNTs enhances the grain size of the perovskite absorber and improves the crystalline structure of the absorber at the interface between the absorber and the HTL. As the grain size increases, the grain boundaries are reduced, thereby eliminating the charge trapping regions within the perovskite structure. The High Voc indicates a low recombination rate where charge accumulation is minimized. However, the variation in the values of Voc in both devices could be as a result of hysteresis effect (Ihly, *et al*., 2016).

The PCE is dependent on the photovoltaic parameters of Jsc, Voc and FF. From the obtained photovoltaic parameters, the PCE increased from 2.82% to 4.30% with an enhancement value of 52.48%. This is due to improved surface area of MWCNTs by acid treatment in the generation of functional group that act as conducting bridge in reducing the contact resistance between individual nanotubes. The ultrasonic process carried out on the MWCNTs reduces their strong agglomeration tendencies due to π - π stacking interactions (Martinez-Rubi *et al*, 2007; Lin *et al*, 1998).

This improved efficiency as a result of the incorporation of MWCNTs, results in better light coupling, exciton dissociation and improved charge transport. The presence of MWCNTs which is hydrophobic in nature, minimizes the creation of humidity sites in the interface between the absorber and the HTL. There is an enhancement leading to high mobility charge pathways and also enables bridging sites for better percolation between the light absorber and the counter electrode.

CONCLUSION

In this research work, two cells were fabricated with the reference cell (without MWCNTs) having device architecture of Glass/FTO/c-TiO_2/m-TiO_2/ZrO_2/$MAPbI_3$/Elcocarb while the second cell incorporated with MWCNTs has device architecture of Glass/FTO/c-TiO_2/mTiO_2/ZrO_2/$MAPbI_3$/MWCNT/Elcocarb. The results show that the incorporated MWCNTs significantly improved the performance of the PVSC by increasing J_{SC}, FF and PCE. The best performing cell incorporated with MWCNTs gave a J_{SC} of 8.47 $mAcm^{-2}$, V_{OC} of 0.85 V, FF of 60 % and PCE of 4.30 %. The cell produced ~ 1.52 times improvement in power conversion efficiency, ~ 1.11 times enhancement in photocurrent density and ~ 1.43 times improvement in fill factor compared to the results obtained with the solar cell without MWCNTs. This study may be of huge benefit for future practical applications and the commercialization of carbon nanotube based PVSCs.

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