Recent Research Advances in Biology

Vol. 5



Book Publisher International

Recent Research Advances in Biology

Vol. 5

Recent Research Advances in Biology

Vol. 5

India ▪ United Kingdom

Book Publisher International

Editor(s)

Dr. Sławomir Borek

Assistant Professor,
Department of Plant Physiology, Faculty of Biology, Adam Mickiewicz University, Poznań, Poland.
Email: borek@amu.edu.pl;

FIRST EDITION 2021

ISBN 978-93-90516-93-3 (Print)
ISBN 978-93-90516-94-0 (eBook)
DOI: 10.9734/bpi/rrab/v5





© Copyright (2021): Authors. The licensee is the publisher (Book Publisher International).

Contents

Preface i

Chapter 1 1-12
Emphasizing the Human Carnivores Conflict in Wamba District, Samburu County, Kenya
William O. Ogara, Nduhiu Gitahi, Alfred O. Mainga and Eunice B. Ongoro

Chapter 2 13-23
The African Blackwood (D. melanoxylon) Seedling Initiation Technicality, (Germinability and Rootability)
Washa B. Washa

Chapter 3 24-41
Phytohormones Production in Microalgae and the Methods of Characterization
Cam Van T. Do, Thuan Dang Tran and Quang Trung Nguyen

Chapter 4 42-55
Liver Enzymes and HIV-infected Adults on HARRT: Evidence from a Randomised Cross-sectional Study in UPTH
Chinwe F. Anyanwu and Eric O. Aigbogun Jr.

Chapter 5 56-65
An Overview of a Neurocosmological Neurotheology Organized around Reissner's Fiber
L. Wile

Chapter 6 66-77
Emphasizing on Reissner's Fiber: A Forgotten Pathway for Exploring Consciousness
Lawrence Wile

Chapter 7 78-94
Influence of Climatic Changes on the Distribution of Geographical Spaces from the Floating Ivory Coast: Case of the Family of Acanthaceae
Ebah Estelle Asseh, Emma Ake-Assi and Kouao Jean Koffi

Chapter 8 95-121
Tidal Influence on Nutrients Status and Phytoplankton Population of Okpoka Creek, Upper Bonny Estuary, Nigeria
O. A. Bubu-Davies and O. A. Ugwumba

Chapter 9 122-127
A Critical Study on Seasonal Variation in Major Minerals (Ca, P, K, Mg) and Proximate Composition in Flesh of Mesopotamian Catfish (Silurus triostegus Heckel, 1843) from Turkey
Mine Perçin Olgunoglu, Ilkan Ali Olgunoğlu and Mustafa Göçer

Chapter 10 128-154
Taxonomic Keys of Mayflies in the Palni and Cardamom Hills of Western Ghats, Southern India
S. Barathy, T. Sivaruban and Pandiarajan Srinivasan

Chapter 11 155-168
HMB Supplementation and Resistance Training: Current Overview on Inflammation, Oxidative Stress and Cardiovascular Risk Factors
Hamid Arazi, Behzad Taati and Katsuhiko Suzuki

Chapter 12 169-174
Isolation of Dermatophytes from Superficial Fungal Infections at Badaun, Uttar Pradesh
Pankaj Saxena

Preface

This book covers key areas of biological research. The contributions by the authors include human-carnivore-conflict, germination, moisture level and non-mist propagation, sprouting, seedling production, tissue culture, phytohormones, microalgae, biofertilisers, microalgal biomass, biofuel production, cyanobacteria, antiretroviral treatment, cholestatic abnormalities, neurotheology, quantum biology, subtle anatomy, quantum mysticism, hepatocellular abnormalities, Reissner's fiber, yoga, biological diversity, climate change, aquatic pollution, environmental surveillance, phytoplankton, anthropogenic effluents, indicator species, endemism, taxonomic primer, oxidative stress, inflammatory markers, cardiovascular risk factors, fungal infection, dermatophytosis. This book contains various materials suitable for students, researchers and academicians in the field of biological research.

Chapter 1
Print ISBN: 978-93-90516-93-3, eBook ISBN: 978-93-90516-94-0

Emphasizing the Human Carnivores Conflict in Wamba District, Samburu County, Kenya

William O. Ogara[1*], Nduhiu Gitahi[1], Alfred O. Mainga[1] and Eunice B. Ongoro[2]

DOI: 10.9734/bpi/rrab/v5/6068D

ABSTRACT

Human-livestock-carnivores conflict is a unifying theme on a complex problem of a shared environment. This makes the interfacing more prominent resulting in increased depredation and cross-species disease transmission with a negative economic impact to involved communities. The study was conducted to determine the levels and nature of human wildlife conflict in Samburu County, Kenya. The data was collected from 2006 to 2009, in three community areas. A total of 266 homesteads were randomly selected for interviews and characterization. The survey was carried out using semi-structured questionnaires, direct field observations and physical measurements. Data obtained was analyzed using Genstat® Discovery (3rd Edition University of Reading), and levels of association established using chi-square. Carnivores within the ecosystem were identified using motion sensor cameras which included lion (*Panthera leo*), leopard (*Panthera pardus*), hyena (*Crocuta crocuta* and *Hyaena hyena*), jackal (*Canis mesomelas*), and wild-dog (*Lycaon pictus*). Livestock predation occurred at different sites notably homestead, water-point and grazing field. A total of 435 cattle, 801 sheep, 1138 goats, 189 donkeys, and 92 camels were killed by carnivores during the study period. The magnitude of depredation was mainly influenced by animal husbandly practices, including poorly constructed animals' sheds and animals left in the field without headers or under the care of minors unable to scare the carnivores away. The dynamics of both wild and domestic ungulates at different seasons are considered to be possible influencing factors on carnivore diet composition, hence presenting as increased conflict during droughts. This study is specific to Samburu County and underpins its relevance and timeliness as pastoralism remains a major source of livelihood in this jurisdiction. This study also shows close interaction between humans and animals which could also be of importance for other dimensions of study in human carnivore studies including a one health dimension.

Keywords: Human-carnivore-conflict; ungulates; homestead; denning site; depredation.

1. INTRODUCTION

1.1 Integrated Ecosystem

Human-Carnivore interface is complex and takes different forms, definitions and levels. Finite competition for resources between wild ungulates and livestock for pastures create opportunities for carnivores to capitalize on easily accessible prey. This makes the interfacing more prominent resulting in increased depredation and cross-species disease transmission with a negative economic impact to involved communities [1,2]. Shared ecosystems have resulted in transmission and resurgence of diseases that were previously under control [3]. The socio-cultural, financial and political challenges that plague management of large carnivores in human-use areas makes it difficult to formulate policies that ensure their conservation while also safeguarding human lives, property, livelihoods and well-being [4,5].

[1]Department of Public Health, Pharmacology and Toxicology, University of Nairobi, P.O.Box 29053-00625, Nairobi, Kenya.
[2]Department of Climate Change, University of Nairobi, P.O.Box 29053-00625, Nairobi, Kenya.
**Corresponding author: E-mail: wogara@gmail.com;*

Large carnivores population has been on the decline globally, a fact attributed to livestock depredation and human development [6]. In Kenya, killing of six lions (*Panthera pardus*) in a single encounter with pastoralists was witnessed at the outskirts of the Nairobi National Park. The big cats strode out of the park to the neighboring farms in search of domestic prey [7]. Livestock attacks by carnivores have been directly related to drought seasons as well as decreased numbers of wild ungulates. A growing human population and new settlements have increased encroachment on wildlife areas additionally increasing human-carnivore conflict [6,2].

1.2 Cultural and Social Habits

The Samburu pastoralists have a philosophy of keeping large herds of cattle. The cattle have more social than economic value. Cattle symbolize prestige and power such that the more herds of cattle one owns, the more recognition, prestige and power one is accorded [8]. Livestock plays a big role in achieving and sustaining social fabric and social capital within the community. Livestock management practices (e.g. level of protection, general condition of stock) also affect conflict [9,10,11]. A lot of social benefits are accrued with this recognition, for instance, marrying many wives. In this regard livestock is used to pay bride price during marriage and also slaughtered during such festivities, strong relations and kinships. Cattle are also used as compensation for collateral damages and for other goods or services rendered [12]. The aspect of cattle keeping is intertwined with peoples' social and economic livelihoods.

The current Samburu way of life has a direct negative impact on environment and natural resources. The many herds of cattle that the community owns deplete the pasture and vegetation, hence enhancing the biophysical impacts such as soil erosion, land degradation and dwindling of water resources [13]. The community gets into conflicts with their neighboring communities over sharing of limited pasture and water resources. The conflicts also involve wild animals that share these limited resources and community attitudes towards wildlife are rapidly changing. There are emerging, managed community conservancies and open community grazing lands that constitute carnivore dispersal areas where human and carnivores interface [14,13].

The objective of this study was to determine the levels and nature of human wildlife conflict in Samburu County, Kenya. The scope of the study included grazing sites, homesteads and carnivore denning sites. The study investigated the nature and levels of conflict as well as the carnivores involved at the different interfaces.

2. MATERIALS AND METHODS

2.1 Study Area

The study was carried out in Samburu County, 400 km north of Nairobi, in community grazing area and sanctuaries of Lodungogwe, Nkaroni, Ngilai west, Namunyak and west Gate (Fig. 1). The main economic activity of the Samburu people is pastoralism with livestock depredation being a major economic constrain [15].

2.2 Homestead Characterization

Livestock bomas (stockades) within the study area were mapped individually and dimensions taken using a tape measure. The following information was recorded: GPS position, date and time, wall height and thickness, construction material, number of compartments in the boma, number and type of stock in the boma, number of people living at the boma, number of dogs present at the boma, number of fires near the boma at night, presence of families living at the boma, duration of time the boma is used, type of habitat surrounding the boma, position of livestock sleeping area in relation to human shelter(s) (Fig. 3). This helped in making determinations on how the homesteads' dynamics influenced depredation particularly at home.

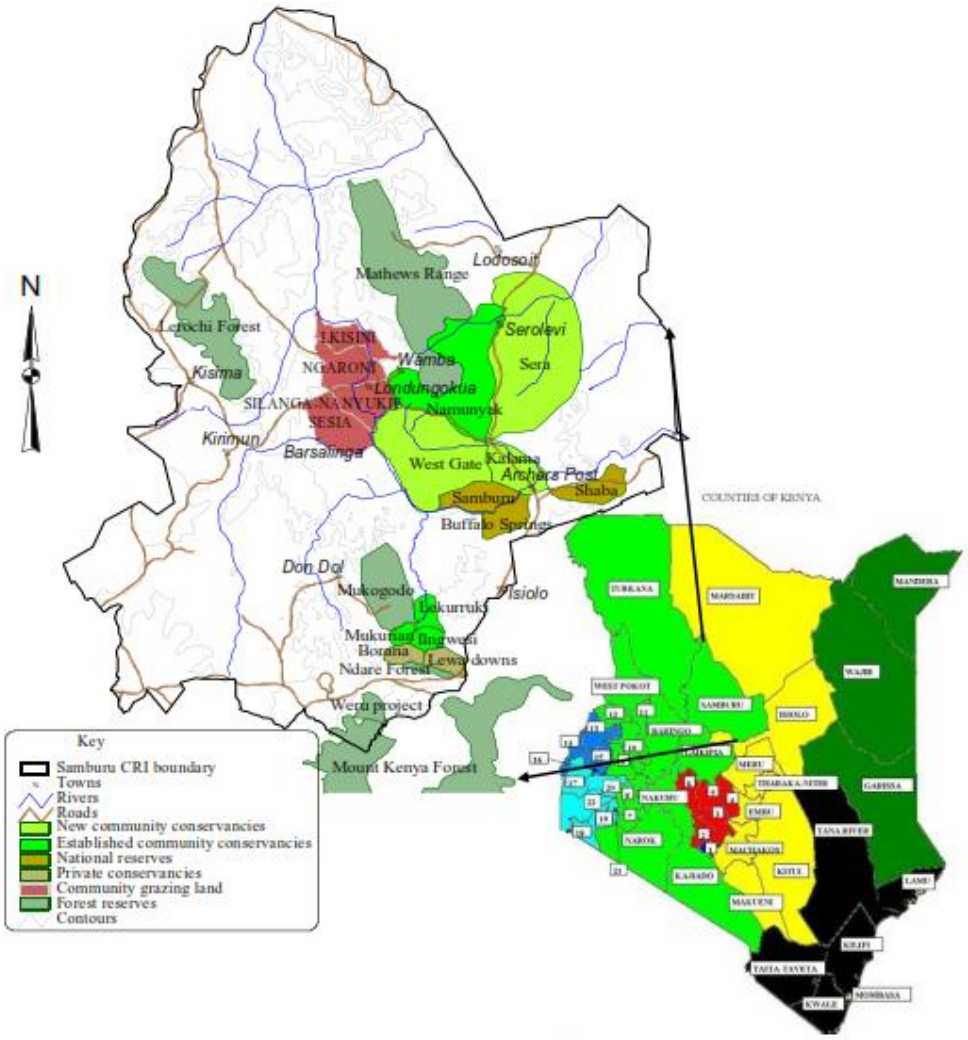


Fig. 1. Map of study area showing the community gracing land, community conservancies and national reserves

2.3 Survey of Predation Incidences (Livestock Kills at Homesteads, Grazing Fields, Watering Points)

We documented the details of predator attacks at each boma. It was necessary to do this for every attack, because circumstances surrounding an attack could vary depending on the habitat, normal practice of the particular group ranch and habits of the herders. Because of the large size of the study area, much of the predation information was obtained from community groups and livestock owners at specific intervals for four years.

2.4 Boma Kills

To establish the magnitude of depredation occurring at home and the problem carnivore (s), the head of the family or the elder present at the time was interviewed at the randomly selected homesteads. For incidents where the predator involved in an attack was not seen, its identity was established by the presence of spoor (tracks) and mode of attack as follows:

Lions' general mode of attack is to roar, stampede cattle and kill outside the boma with a bite at the neck. Identity confirmed by tracks and distance between canine teeth on the bite mark. A lion cannot scale boma walls more than 2 m high. Lions usually move in prides.

Leopards are solitary hunters and rely on stealth to catch their prey. Leopard usually prey on animals of a size that they can easily climb a tree with or drug into a thicket after capture.

Subsequently shoats, young calves and dogs form a greater part of their prey.

Hyenas usually force their way under or through perimeter fence. Their tracks are easy to identify because of a distinctive pad shape (Personal communication; Samburu elders).

2.5 Field Kills

Livestock attacked and killed while grazing were considered separately as another form of wildlife livestock interaction. This was because such attacks had a strong component of vigilance problem, and consequently, factors such as stock to herder ratio and presence of dogs assume increased importance. Such attacks also involved other species such as cheetahs (*Acinonyx jubatus*), jackal (*Canis mesomelas*), and wild-dog (*Lycaon pictus*), which rarely attack at bomas. The following information was recorded for field kills: Herd size, number of predators involved in the attack, number of herders present during the attack, type of habitat, number of dogs, location and time of the incident, distance between herder and livestock. This information was obtained from interviews with the herders in charge of the attacked herd. Animals killed because of being left out in the field at night were recorded separately to measure the impact of herding practices and overall loss.



Fig. 2. A typical Samburu homestead as observed in the study area

2.6 Motion Sensor Cameras Studies

Motion sensor cameras were placed near human settlements, livestock stockades, dens, at kills and left-over carcasses, commonly used watering points and places where locals often spot carnivores. The cameras recorded times of day or night when pictures were taken. Motion sensor cameras provided added accuracy to the identification of individual animals for instance differentiating between striped and spotted hyenas which was not possible using spoors and feeding style.

3. RESULTS

3.1 Homestead Characterization

Two hundred and sixty-six homesteads were characterized. The majority, 88.4%, had perimeter fences and animals' sheds constructed with acacia branches while the rest (11.6%) had thorny shrubs found in the study area as well as shrub fences. Most of the houses (95.5%) were thatched with grass and cow dung. Other roofing materials were used but to a lesser extent, including nylon and plastics (3%), animal skin (1.1%) and corrugated iron sheets (0.4%).

The average height of perimeter fences at the homestead was 102.4 cm, with an average thickness of 114.5 cm. There was an average of 3 dogs per homestead (range: 0-7). A mean of 275 livestock, including cattle and shoats, were recorded in each of the characterized homesteads.

Homesteads were located at an average distance of 5.76 km to the nearest water source(s) and 3.68 km to river valleys. Most were dry depending on the season. Most of the homesteads were located close to inselbergs and linear ridges with an average distance of 1.87 and 2.64 km respectively, however most homesteads were at an average of 8.85 km far from mountain ranges. Settlements were found to be strategically located, with an average distance of 1.14 km between homesteads, with most of them about a kilometer from thick bushes.

3.2 Survey of Predation Incidences (Livestock Kills at Homesteads, Grazing Fields, Watering Points)

Numbers of domestic animals preyed on by each predator over the four years of study are shown in Fig. 3. Goats (41.7%, n = 1138) were the most preyed on domestic species having been killed by all the carnivores. The highest number of goats (23.1%, n = 263) were killed by the leopard, while the crocodile (0.26%, n = 3) and the lion (8.9%, n =102) recorded the least goat kills respectively. Camels (3.4%, n = 92) were the least predated on among domestic ungulates by all the predators, with the lion recording the highest camel kills (84.4%, n = 74). The lion (22.5%, n = 615) and hyena (22.4%, n = 612) were on the other hand the most nuisance carnivores having depredated on the highest number of domestic ungulates, followed by the leopard at (19%, n = 521. Crocodile killed the least (0.2%, n = 6). Most carnivores had a preference for goats followed by sheep except the lion that preferred cattle. This could be attributed to the population dynamics of the domestic ungulates in the study area as well as the size on edible meat in the different species and ease of kill (Fig. 4).

The total number of domestic ungulates killed at the watering point were the lowest (4.5%, n = 124) compared to the homestead (21.4%, n = 584) and the grazing field (49.9%, n = 1363), where half of all the kills were recorded to have taken place. Amongst all the predators the jackal had the highest number of kills at the homestead (33.6%, n = 196) followed by the eagle at (23.5%, n = 137). The two predators, jackal and the eagle prefer the young of the small stock that are grazed near the homesteads. Amongst the carnivores found in the study area, the hyena had the highest kill (15.4%, n = 90) while the lion killed the least (4.8%, n = 28) at the homestead. Analysis carried out indicated a strong association between all the predators and kills at the homesteads (Fig. 5).

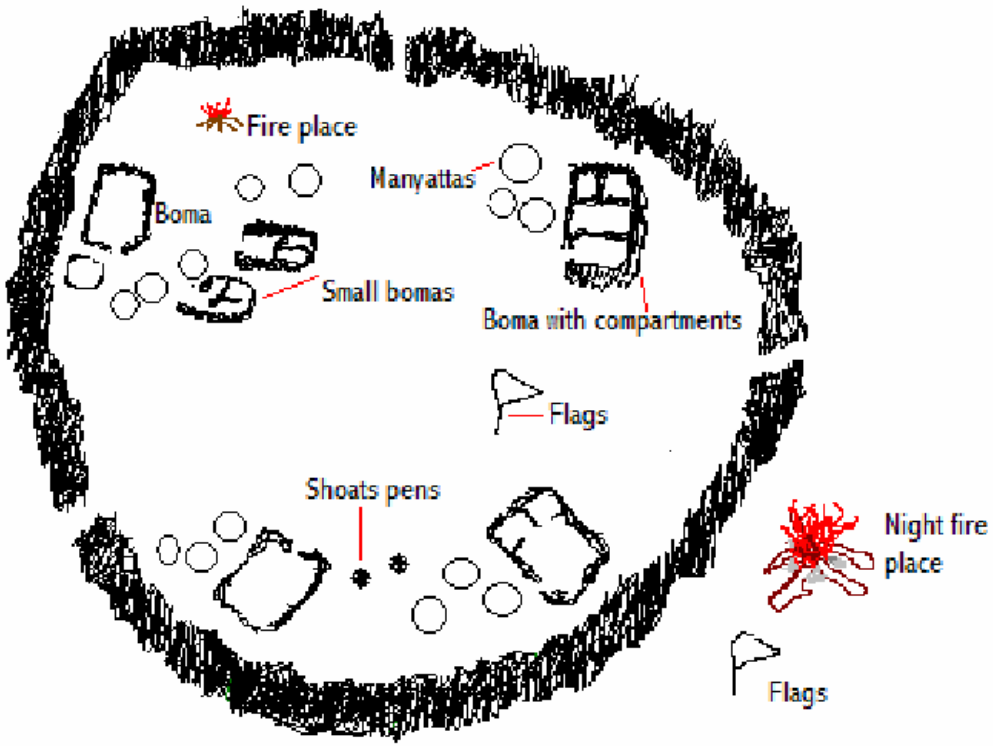
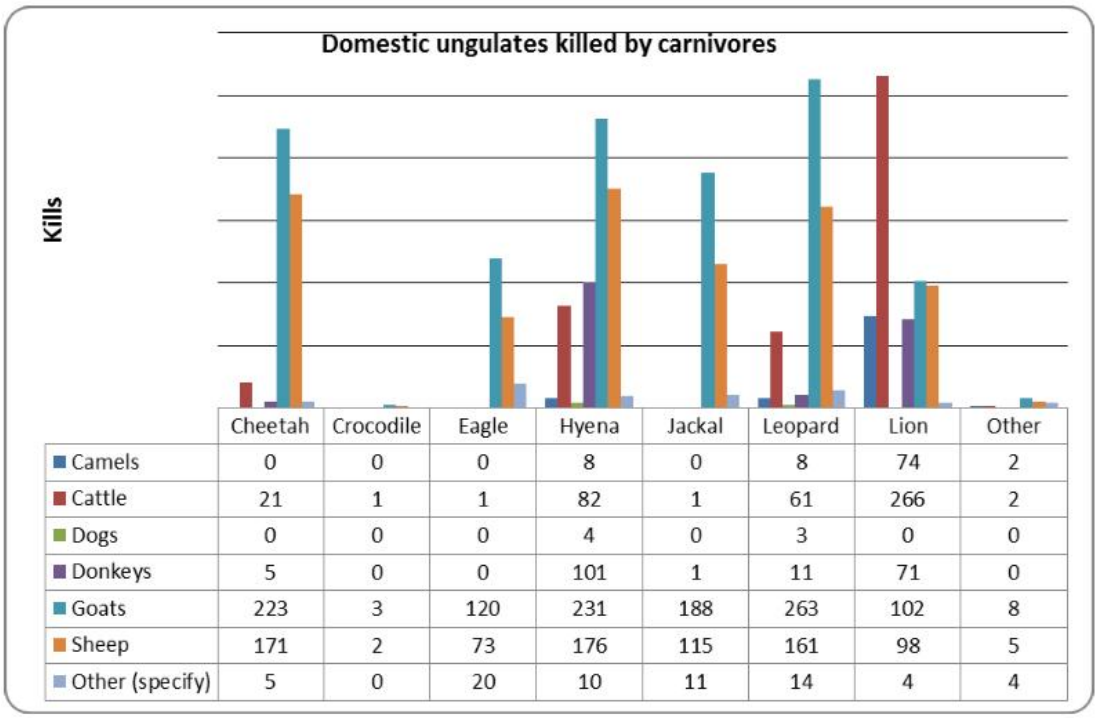
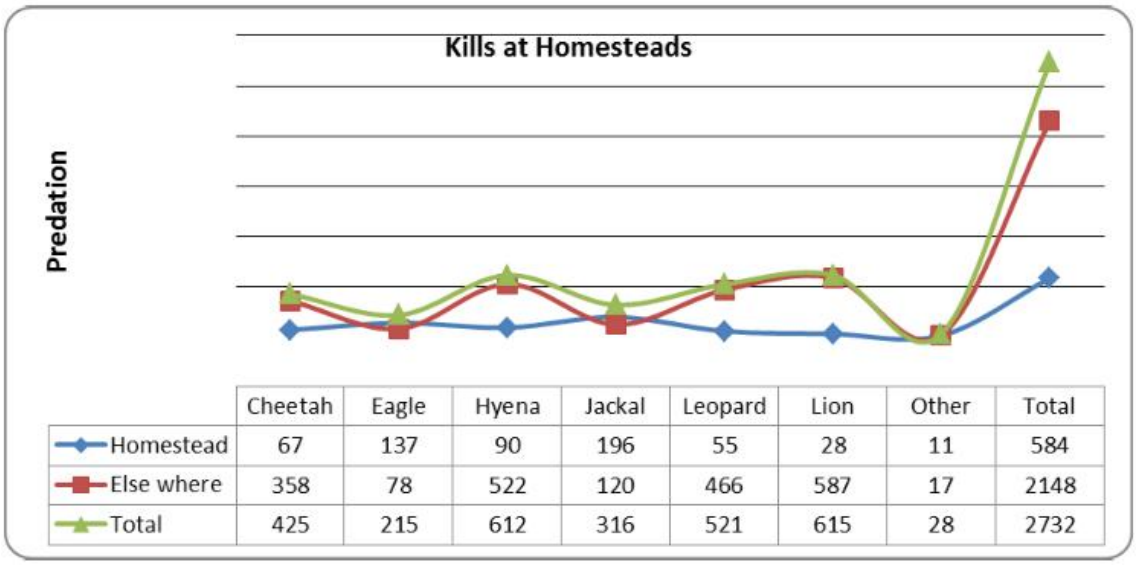


Fig. 3. A typical homestead showing parameters characterized at the study area



	Cheetah	Crocodile	Eagle	Hyena	Jackal	Leopard	Lion	Other
Camels	0	0	0	8	0	8	74	2
Cattle	21	1	1	82	1	61	266	2
Dogs	0	0	0	4	0	3	0	0
Donkeys	5	0	0	101	1	11	71	0
Goats	223	3	120	231	188	263	102	8
Sheep	171	2	73	176	115	161	98	5
Other (specify)	5	0	20	10	11	14	4	4

Fig. 4. Showing the magnitude of losses during study period (four years) through depredation by the different types of carnivores in the study area



	Cheetah	Eagle	Hyena	Jackal	Leopard	Lion	Other	Total
Homestead	67	137	90	196	55	28	11	584
Else where	358	78	522	120	466	587	17	2148
Total	425	215	612	316	521	615	28	2732

Fig. 5. Shows predation of domestic ungulates by different carnivores at the homesteads

At the watering point it was shown that the lion recorded the highest kill at (42.7%, n = 53, followed by the leopard (16.1%, n= 20 while the jackal killed the smallest number (3.2%, n = 4 of domestic ungulates. There was a strong association of the lion with watering point as a location of kill ($P<0.05$) and chi square > 3.84.

The lion (32.7, n = 446) and the cheetah (23.2, n = 316) killed most of the domestic animals at the grazing field during the study period respectively, while the eagle and jackal recorded the least kills at (2.3, n = 32 and (4.8%, n = 66 respectively (data not shown).

3.3 Motion Sensor Cameras Studies

Motion sensor cameras mounted at different carnivore den sites as well as areas frequented by the predators provided added accuracy to the identification of individual animals. At some instances, the striped and spotted hyenas were shown to exist at the study area but at different den locations (Fig. 6). Other carnivores captured by the cameras were the leopard and jackal. It was also possible to show that in some instances the carnivores shared their denning areas with other animals, most notably the porcupine (*Hystrix ristata*).

3.4 Carnivore Den Sites Characterization

A total of 104 carnivore dens were identified at the study area, (59.6%, n = 62) were actively in use while (37.5%, n= 39) had no resident carnivore at the time of characterization. Most dens were located at inselbergs with an average height of 165.5 m. More than half (65.8%, n = 48) of the dens had not been visited by human beings, since they had no visible human activity, whereas (23.3%, n=17) of the dens had minimal human activity. A small number (11%, n = 8) of the dens showed pronounced human activity marked by presence of fire places and food leftovers with the cave rocks' colored with the morans' hair dyes.

Apart from the resident carnivores, other animals mostly wild ungulates were reported at the den sites, these include; dikdik (51.6%, n = 16), rock hyrax (12.6%, n = 4), gerenuk (12.9, n = 4), clip springer (9.7%, n = 3) and gazelles (6.5%, n = 2). There were indications that two cheetahs were also cited at the den site throughout the study period though they were not the resident carnivore. Pictures are important in determining habitat used by wild carnivore species, and appropriate livestock husbandry practices may be developed.

4. DISCUSSION

Increased livestock population, climatic factors, land degradation, increased human population continue to cause an imbalance in the Samburu ecosystem where carnivores, humans and livestock no-longer co-exists harmoniously [16]. The co-existence has thus resulted to increased conflict bearing in mind that more than 50% of wildlife are in community grazing area and community group ranches [17]. In the study area, the entire homestead sampled had perimeter fence, made of thorny branches, in most cases from the acacia tree. The homesteads were desired to have separate animal sheds for shoats, cattle, camel and donkeys. Human dwelling houses were specifically located on the periphery, with most vulnerable animal (shoats) positioned at the center while donkeys which were also regarded as guard animals had a shed next to the perimeter fence (Fig. 2). On average the perimeter fence was 102 cm high and 114 cm thick. In most cases the fence was not an effective barrier for keeping the carnivores away. This was due to decay and degradation of the fences that was observed particularly during droughts. Similar observations on use of poorly maintained acacia fences as homestead perimeter fences that could not keep carnivores away have been made by other authors [16,18].

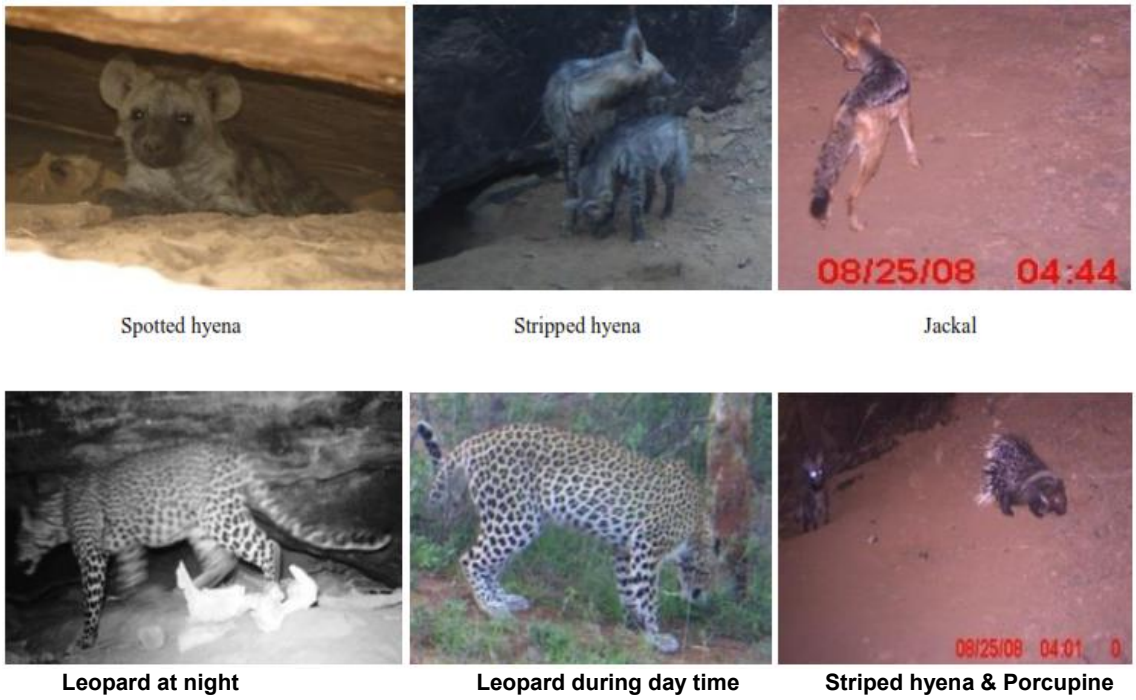


Fig. 6. Showing images of carnivores and other animals sharing den sites captured at the study area

All the carnivores identified in this study were shown to have a strong association with homestead as a livestock killing site, ($P<0.05$), the hyena having the highest night incidents at the homesteads. This was attributed to poor fencing of animal sheds in the study area. Better fencing technology should be encouraged in the study area to reduce preventable conflicts. Such predator proof mobile bomas have been tried elsewhere with very encouraging results. A 91.1% reduction in livestock predation during the night as a result of using the predator proof bomas at Amboseli, Kenya has been reported [19].

Dogs were kept in most homesteads with an average of 3 dogs per homestead. Some homesteads however had as many as seven while others had none. Though dogs were kept as guard animals, kills at the homesteads still occurred at night mostly by the hyena; (15.4%, n = 90) and the jackal killed; (33.5%, n = 196) young shoats left at the homestead under the care of young children. The effectiveness of the dog as a guard animal greatly depends on how the animal was trained and

bonded with livestock. The Samburu people have been using the dog to chase predators away and also alert the pastoralist of their presence. There are instances where dogs have been preyed on by leopards [18,16].

Location of homesteads was observed to be strategic, and was mostly influenced by proximity to water sources, grazing fields and shrubs for the browsers. The convenience of these essentials was also found to be favored by wild ungulates and by extension the carnivores. Characterization of carnivore denning sites showed that hyenas and leopards preferred mountain ranges and inselbergs. This close co-existence could be attributed to the observed human–carnivore conflict in the study area, an observation that was also made by Okello [18], who attributed increased depredation to livestock encroachment to carnivore habitat. The situation is worse during droughts when livestock are moved to carnivore ranging zones in search for pasture, hence becoming the primary prey [2].

5. CONCLUSIONS

The phenomenon of human carnival conflict is something that will be there as long as human beings, livestock and carnivores continue to share the same habitat. This therefore calls for sustainable policies that can guide and manage the three to create a harmonious relationship that is sustainable. Predation on livestock is a major factor in human wildlife conflict in Samburu. Significant off-take was reported in this study. The conflict is compounded by the habitat characteristics, community practices including shelter, management of herds both at night and during the day. The impact of the off-take by carnivores is significant and definitely impacts the Samburu pastoral area as a source of meat for the Kenyan market and their livelihood. The study also demonstrates that the problem of human carnivore conflict is complex and real. The effective management of the conflict for better results including economic and social outcomes requires paradigm shift supported by strong policy decisions and management of community perceptions and practices. Homestead and livestock shelter construction stand out as contributors to off takes at night. Devolved government is getting grounded in Kenya and County based studies should generate keen interest and contribute to policy and practice for sustainable livelihoods. Policy on compensation should be enacted to enable compensation for loss of livestock to wildlife. This will lessen the conflict by reducing retaliatory practices and ultimately creating a positive attitude towards the problem carnivores. The County Government together with the involved communities needs to embrace the aspect of ecotourism within the conservancies, thus best managing their resources including wildlife, land and livestock.

This study is specific to Samburu County and underpins its relevance and timeliness as pastoralism remains a major source of livelihood in this jurisdiction. This study also shows close interaction between humans and animals which could also be of importance for other dimensions of study in human carnivore studies including a one health dimension. Diseases at the human-animal interface is an area of concern with specific attention to emerging and re-emerging diseases as demonstrated by [20].

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Kingdon K. The Kingdon field guide to African mammals. ISBN 0-7136-6513-0, 3rd ed. Christopher Helm Publishers, London WID 3QZ; 2003.
2. Treves A, Karanth KU. Human-carnivore conflict and perspectives on carnivore management worldwide. Conserv. Biol. 2003;17:1491-1499.
3. Amy B, Pedersen, Jonathan DT. Cross-species pathogen transmission and disease emergence in primates. EcoHealth. 2010;6(4):496-508.
4. Chapron G, Lo´pez-Bao JV. Conserving carnivores: Politics in play. Science. 2014;343:1199–1200.

5. Srivathsa A, Puri M, Karanth KK, Patel I, Kumar NS. Examining human–carnivore interactions using a socio-ecological framework: Sympatric wild canids in India as a case study. Royal Society Open Science. 2019;6(5):182008.
6. Kolowski JM, Holekamp KE. Spatial, temporal, and physical characteristics of livestock depredations by large carnivores along a Kenyan reserve border. Biol. Conserv. 2006;128:529-541.
7. BBC News. Six lions killed in Kenyan capital Nairobi Six lions are killed on the outskirts of Nairobi after straying into a residential area; 2012.
Available:www.bbc.co.uk/news/world-africa-18522190
8. Spencer P. The Samburu: A study of Gerontocracy in a Nomadic Tribe. London: Routledge and Kegan Paul; 1965.
9. Hoogesteijn R. Manual on the problem of depredation caused by jaguars and pumas on cattle ranches. Wildlife Conservation Society, New York; 2003.
10. Ogada MO, Woodroffe R, Oguge NO, Frank LG. Limiting depredation by African carnivores: The role of livestock husbandry. Conservation Biology. 2003;17:1521–1530.
11. Zimmermann A, Baker N, Linnell JDC, Inskip C, Marchini S, Odden J, Treves A. Contemporary views of human-carnivore conflicts on wild rangelands. Can Rangelands be Wildlands? Wildlife and Livestock in Semi-arid Ecosystems. 2010;129-151.
12. Sobania N. Herders: Subsistence survival and cultural change in Northern Kenya. J. Afr. Hist. 1988;29(1):14-40.
13. Lamprey H. Pastoralism yesterday and today: the overgrazing problem. In Tropical Savannas: Ecosystems of the world. Edited by: Bourliere F. Amsterdam: Elsevier. 1983;643-666.
14. Hardin G. The tragedy of the commons. Science. 1968;162:1243-1248.
15. Ogara WO, Muchemi G, Oguge NO, Olesarioyo JS. People, livestock, and wildlife: Existing best natural resource management practices in Naibung'a and Namunyak Community Based Conservancies; 2011.
16. Ocholla GO, Koske J, Asoka GW, Bunyasi MM, Pacha O, Omondi SH, Mireri C. Assessment of traditional methods used by the Samburu pastoral community in human wildlife conflict management. Int. J. Human. Soc. Sci. 2013;3(11):292.
17. Kameri-Mbote P. Land tenure, land use and sustainability in Kenya: Towards innovative use of property rights in wildlife management, (IELRC Working Paper, Geneva Switzerland). 2005;14-18.
18. Okello MM, Bouham R, Hill T. The pattern and cost of carnivore predation on livestock in Masaai homesteads of Amboseli ecosystem, Kenya: Insights from a carnivore compensation programme. Int. J. Biol. Conserv. 2014;6(7):502-521.
19. Manoa DO, Mwaura F. Predator-proof bomas as a tool in mitigating human-predator conflict in Loitokitok Sub-County, Amboseli Region of Kenya. Nat. Resour. 2016;7:28-39.
20. Owange NO, Ogara WO, Gathura PB, Okuthe S, Mbabu M. Occurrence of rift valley fever in cattle in Ijara district, Kenya. Prev. Vet. Med. 2014;117:121-128.

Biography of author(s)



Professor William O. Ogara
Department of Public Health, Pharmacology and Toxicology, University of Nairobi, P.O.Box 29053-00625, Nairobi, Kenya.

He has special interest and experience in transdisciplinary research, working with team members from different academic and science backgrounds. He has strengthened wildlife studies and research in the Faculty of Veterinary Medicine, University of Nairobi with a focus in human wildlife conflict building into One health Studies and a member of Planetary Health. His research areas include food safety and value chain, environment, Climate change and health, human wildlife conflict challenges. He has published over 60 papers in refereed journals. He has received awards and recognition by the veterinary profession in Kenya for transformative leadership and development of the profession locally and internationally. He applies the broad base transdisciplinary experience as a lead expert in Environmental and Social Impact Assessment. He has outstanding administration and academic leadership at the University, as Chair of department and Director of international programmes and Links.



Dr. Nduhiu Gitahi
Department of Public Health, Pharmacology and Toxicology, University of Nairobi, P.O.Box 29053-00625, Nairobi, Kenya.

He holds a Doctor of Philosophy (PhD) in Immunology and complete M.Sc. from Kenyatta University. Currently, he is the Principal Technologist in charge of laboratories in the Department of Public Health, Pharmacology and Toxicology, University of Nairobi. He has over 13 years of professional experience in Laboratory Consultancy and has supervised 4 MSC students drawn from Kenya and Sweden. He has 26 publications in peer reviewed journals. He is involved in teaching Undergraduate and postgraduate students on Analytical Techniques, Applied immunology, Quality control, Hazard analysis and critical control points. His research interests are in the area of bacteriology and molecular immunology. He has worked with research projects in the areas of human waste management, food safety, water quality, antimicrobial resistance, and human wildlife conflict and consulted on various aspects of food safety and domestic water quality. He participated in a study on the state of pollution of Nairobi River basin and Lake Victoria and its main catchment rivers with the Nation Media Group. He is currently a Co-PI on a project funded by the Engineering and Physical Science Research on 'addressing livestock derived antimicrobial pollution in the Nairobi River in Kenya'.



Mr. Alfred O. Mainga

Department of Public Health, Pharmacology and Toxicology, University of Nairobi, P.O.Box 29053-00625, Nairobi, Kenya.

He is a Senior Technologist in the Department of Public Health, Pharmacology and Toxicology, Faculty of Veterinary Medicine in the University of Nairobi. He holds a Diploma in Medical Laboratory Science (Technical University of Kenya 2001-2004), Bachelor of Science degree in Medical Laboratory Sciences (Mt. Kenya University 2011-2013) and Master of Science degree in Veterinary Science (Paraclinical Sciences) from the University of Pretoria South Africa (2016-2017). His Master of Science

degree research project was on Occurrence and characterisation of the seven major Shiga toxin-producing Escherichia coli (STEC) serotypes in healthy beef cattle in South Africa. While at the University, he has worked as a Field and laboratory Research assistant with projects, Health Risk Analysis of Cryptosporidiosis in Urban Small Dairy Production in Nairobi and Carnivore in Conflict with Livestock in Samburu. He has also worked in a number of postgraduate and PhD students' research projects on health and hygiene, milk/meat/water bacteriological quality and hygiene, medicinal plant extracts efficacy and antimicrobial resistance (phenotypic and genotypic), mycotoxins analysis and co-authored in several published articles in renown journals. His main interest and potential is in phenotypic and genotypic characterization of zoonotic pathogens of public concern and surveillance of resistance and virulence genes.



Eunice B. Ongoro
Department of Climate Change, University of Nairobi, P.O.Box 29053-00625, Nairobi, Kenya.

She holds a Bachelors and Master's degrees from the University of Nairobi. She is currently a doctoral candidate in the same University, her research and thesis is focusing on food security and agricultural technologies. Since 2013, she has been teaching and conducting research at the Institute for Climate change and Adaptation of the University of Nairobi as an associate and later as a tutorial and research fellow. Since 2013 to present at the Institute for Climate Change, she has been involved in many research projects on climate change and conflicts in Northern Kenya, Green Economy mainstreaming across the Kenyan counties, climate change and food security amongst others. Her current research interest is focus on climate change adaptation, gender, livelihoods and food security. She has co-authored several research papers and a book chapter with other researchers published in international journals, she has also presented her research in both international and local conferences.

© Copyright (2021): Author(s). The licensee is the publisher (Book Publisher International).

DISCLAIMER
This chapter is an extended version of the article published by the same author(s) in the following journal.
International Journal of Biodiversity and Conservation, 9(9): 284-291, 2017.

Chapter 2
Print ISBN: 978-93-90516-93-3, eBook ISBN: 978-93-90516-94-0

The African Blackwood (*D. melanoxylon*) Seedling Initiation Technicality, (Germinability and Rootability)

Washa B. Washa[1*]

DOI: 10.9734/bpi/rrab/v5/1603C

ABSTRACT

A study was conducted in *Dalbergia melanoxylon* (African Blackwood) comparing the ability to form seedling between seed germination in the soil, germination in the Murashge and Skoog medium (MS) and rooting of the cuttings to form seedling. An overharvested species *D. melanoxylon* have a highly valued wood but not propagated. This is why in recent years there have been some efforts to conduct investigations which can improve seedling production. About 2 kilograms of seeds were purchased from TTSA and cuttings (root, softwood and hardwood stem cutting) were collected from the forest for experiment on 2010 at the Botany Department University of Dar es Salaam. Three treatments were employed for soil germination (Low, Median and High moisture level) while two treatments were employed for MS germination (Half strength and Full strength). Sterilizing reagents were 35%, 70% alcohol and 2.6% sodium hypochlorite. Sterilizing duration were 10, 20 and 30 minutes. Softwood, semi-hardwood, hardwood and root cuttings were introduced in a non-mist propagator using soil inoculated with mycorrhizae. Data recorded include germination percentage, moisture level, MS concentration, sterilizing reagent concentrations and time used to sterilize the seeds, temperature and humidity in the propagator and sprouting percentage of cuttings. Standard procedures were used to analyze and compare germination and rooting data as described by Zar, [1]. Highest germination in the soil was 21% while that of the MS was 19.8%, rooting was 100% in softwood cuttings and 37% in root cutting while semi-hardwood and hardwood cutting didn't root at all. The two germination mediums (soil and MS) did not differ significantly while using cuttings imply harvesting of the existing forests which is not recommended. More investigations especially genetic transformation of the species for easy access in tissue culture is needed to improve seedling production of *D. melanoxylon* for propagation of the species.

Keywords: Dalbergia melanoxylon; african blackwood; cuttings; rooting; murashige and skoog; germination; moisture level and non-mist propagator.

1. INTRODUCTION

1.1 *Dalbergia melanoxylon* Plant

Dalbergia melanoxylon Guill & Perr is a flowering plant that belongs to Phylum Tracheophyta, class Magnoliopsida, Order Fabalies, Family Leguminoseae and sub-family Papilionoidea. It is also known as African Blackwood and Zebrawood (in English), African ebony (in Mozambique) and Mpingo (in Swahili) [2]. *Dalbergia melanoxylon* wood is highly valued because it is used in wood wind instruments, clarinets, oboes, and pipes. *Dalbergia melanoxylon* is a native to dry regions of Africa. The Genus *Dalbergia* yields other famous timbers such as rosewood, tulipwood and cocobolo (Bekele, 2007). As a member of Papilionoidea species *D. melanoxylon* seeds are usually hard and with a complex hilar valve and enclosed in pods (legumes), symmetrical (Zygomorphic), Perfect or hermaphroditic (bisexual), entermophilous (insect pollinated) and white plants are shrubs or trees (habits), leaves are usually compound and alternate, flowers are usually bilaterally [3].

[1]*University of Dar es Salaam – Mkwawa College (MUCE), P.O.Box. Private Bag, Iringa, Tanzania.*
**Corresponding author: E-mail: wbugalama@yahoo.com;*

1.2 Status of *Dalbergia melanoxylon* in Tanzania

The IUCN Red List [2] uses three categories to evaluate conservation status of plant species (Lower Risk, Threatened and Extinct category). Lower Risk spp may be the Least concern (Lc), near threatened (nt) and Conservation dependent (cd). Threatened spp may be vulnerable species (Vu), endangered species (EN) and critically endangered (CR) and the extinct spp may either be Extinct in the wild (EW) or Total Extinct (EX). *Dalbergia melanoxylon* in Tanzania is classified as Lower Risk / near threatened meaning that it is neither endangered nor of least concern. The current conservation status of *Dalbergia melanoxylon* shows that is threatened in Kenya, extinct in Burkina Faso and therefore need conservation attention in Tanzania [3].

1.3 Geographic Distribution and Natural Habitat of *Dalbergia melanoxylon*

The species is wide spread in tropical Africa from Senegal and Cote d'Ivore in the West, to Kenya and Ethiopia in the East, and extending south to South Africa. It is found in at least 26 sub -Saharan countries [4]. Apart from Africa, *Dalbergia melanoxylon* is also found in India around Pune City [5]. In Tanzania, *Dalbergia melanoxylon* is abundantly found in T4, T5, T6 and T8 floristic regions and less abudant in T1, T2, T3 and T7 floristic regions [6]. *Dalbergia melanoxylon* inhabit rainforest and open miombo woodlands of Tanzania in which rainfall is often marginal with long drought periods. Such areas are environmentally fragile and species vitality depends on a delicate balance between populations within them. *Dalbergia melanoxylon* has adapted well to this marginal habitat and its long growing period is an outcome of this adaptation to harsh conditions [3]. *D. melanoxylon* grows under a wide range of conditions including semi-arid, sub-humid and tropical lowland areas. It is often found on dry, rocky sites but is most frequent in the mixed deciduous forests and savannahs of the coastal region. This species demands water and light and therefore will not regenerate under heavy cover. Mature trees are fire tolerant [3].

1.4 Growth Characteristics and Biophysical Limitations of *Dalbergia melanoxylon*

Dalbergia melanoxylon growth is naturally slow [3]. It thrives well from sea level to 1200m altitude with an average temperature of between 18°C and 35°C and 700 mm to 1200mm of rainfall. The species grows in soils varying from loamy sand to vertisols "black cotton soil" [4]. The species is able to withstand regular fires and hence mature trees can be damaged but usually not killed by bush fires [4].

1.5 Rooting and Rooting Ability

Rooting is the formation of root that arises from the plant cutting or is the formation of part of plant body that bears no leaves and lacks nodes or is the formation of plant organs that lies below the soil surface. Normally a germinating seed need less physiological process for the root to arise from the embryo than physiological process needed for the root to arise from the plant cuttings in which more specialized process are needed. Rooting ability is the ability of the plant body or cutting to raise a certain number of roots and of certain height and widths in a given time. Rooting ability differs in different plant species and differs with types of cuttings, differs with age of the cuttings. Root, stem and leaves cuttings may have different rooting ability within the same species or different species. Both internal (genetically) and external (environmental) conditions are the key factors for rooting to occur or not to occur [7].

1.6 Plant Cuttings and Cutting Types

A plant cutting is a piece of plant body from which roots or stems or leaves will arise when this cutting is kept under suitable or optimal conditions especially the moist soil. Under suitable conditions a piece of plant body will begin to grow as a new plant independent of the parent plant under the process known as striking where by a stem will form roots and a root will form a stem while leaves cutting will form both roots and stems. There are basic three types of plant cuttings (root, stem, leaf) which can be grouped into three classes which are softwood cuttings coming from stems that are rapidly expanding, with young leaves. In many species, such cuttings form roots relatively easily. Secondly are semi-hardwood cuttings coming from stems that have completed elongation growth and have

mature leaves and contain semi-lignified xylem tissues. Finally is the hardwood cuttings coming from fully matured stems, and are often propagated while dormant, they contain fully lignified xylem tissue [7,8].

1.7 Propagation Efforts of *D. melanoxylon* in Tanzania

As an effort towards propagation of *D. melanoxylon* in Tanzania, the African Blackwood Conservation Project (ABCP) was established in 1996 by James Harris, a woodworker from Texas, USA, and Sebastian Chuwa, a botanist from Tanzania [9]. Its purpose was to replenish the population of the African Blackwood, to sensitize the community on economic and ecological importance of the species, to sensitize the community on seedling production for propagation of the species, to conduct research and keep records of their findings. Efforts by ABCP used natural regenerative method (seed germination in soil) which is associated with low regenerative ability and therefore efforts were not successful. Because of the extensive use of this wood for carvings, instrument and woodworking trades, its supply have been decreasing due to exploitation at an unsustainable rate [9]. Although the tree grows in other parts of Africa, the major supply is from Tanzania and Mozambique, countries which have ideal climatic conditions and a characteristic suitable to the specific uses of the wood materials for instrument and carving trades. Trees from other areas lack some of the special qualities (eg tissue packing, color and density) that makes the wood materials not ideal for commercial enterprises ([9]. Because of over exploitation from its natural habitat, the tree is now commercially depleted in Kenya and in some areas of Tanzania. If present exploitation continued with no attempts to replant the tree, geographic areas that are practically devoid of the tree will increase, thus adversely increasing conservation concern in the East African ecosystem in which it grows and affecting numerous commercial enterprises throughout the world. [9]. The ABCP propagation efforts were not based on advanced seedling production techniques but were based on seed germination which was limited by low seed viability and germinability. *D. melanoxylon* has not been cultivated extensively.

1.8 Previous and Recent Propagation Studies on *D. melanoxylon* in Tanzania

The IUCN, [2] reported that *D. melanoxylon* on one side have low regeneration ability while in the other hand is over harvested such that it is threatened in African countries like Kenya and categorized as Low/Risk near threatened in Tanzania. Previously, studies by Redhead and Temu [6], Nshubemuki [4], Sharman [10], TTSA [11] and Msanga, [9] reported low seed viability of less than 30% in *D. melanoxylon* which is also associated with low seed germination and that the growth rate of the species is 70 to 100 years to attain a harvestable age. These reports are supported by recent studies conducted by Amri, [12], Amri, [13]. A study conducted by Amri, [12] reported that *Dalbergia melanoxylon* has low seed viability, seed germination and seedling growth rate. In a related study seed viability varied with different time of seed harvesting, lo w seedling growth rate was also observed On the other hand, Amri, [13] reported an increased rooting ability of 70% stem cuttings using root promoting hormones (IBA). All these studies did not employ the advanced plant - production techniques such as tissue culture in *Dalbergia melanoxylon* as already pointed out by Redhead and Temu, [6]. Although micropropagation has not been conducted on *D. melanoxylon*, investigations have been done in Tanzania and other countries to improve cassava, sisal, cashew nuts, banana and other crops by micropropagati This study intended to test if *D. melanoxylon* seed germination in tissue culture (MS) can exceed that natural germination in the soil.

1.9 Objective of the Research

To assess the effective means of seedling production in the African Blackwood between soil, Murashige and Skoog (MS) seed germination and the cuttings.

2. MATERIALS AND METHODS

2.1 *Dalbergia melanoxylon* Seed Germination in MS

A total of 2 kilograms of *D. melanoxylon* seeds were purchased from TTSA on January 2010 for MS and soil germination experiments. These seeds were 62% tested viable by tetrazolium test. A total of

500 seeds were used in this experiment in 10 replicates having 50 seeds each replicate. The purpose was to compare full and half strength Murashige and Skoog (MS) in effecting seed germination. About 150 seeds were sterilized in 70% ethanol for 30 minutes and 2.6% sodium hypochlorite for 30 minutes and inoculated in full strength MS at pH 5.8. Another 100 seeds were sterilized in 35% ethanol for 20 minutes and 2.6% sodium hypochlorite for 20 minutes and inoculated in half strength MS at 5.8. Other 150 seeds were sterilized in 70% ethanol for 10 minutes and 2.6% sodium hypochlorite for 10 minutes and inoculated in full strength MS at pH 5.8 while the last 100 seeds were sterilized in 35% ethanol for 10 minutes and 2.6% sodium hypochlorite for 10 minutes and inoculated in half strength MS at 5.8 pH. The cultures were incubated in a culture room at 28oC under a photoperiod of 16 hours at 3000 lux light intensity provided by cool white fluorescent tubes. Germination was monitored for 4 months between January to April 2010.

2.2 *Dalbergia melanoxylon* Seed Germination in the Soil

The experiment was arranged in a split plot design with seed soaking and non soaking treatments as the main plots and moisture level in the potting media as subplots. This was followed by observations on seed germination. A total of 360 seeds were soaked in water for 6 hours before sowing while another total of 360 seeds were sown without prior soaking treatment. Each of these 2 main treatments was then subdivided into three sub treatments that were assigned to 3 moisture level treatments namely low, median and high moisture in the potting mixture. The topsoil part of the potting mixture was obtained from Kunduchi quarry so that a recommended ratio of potting media can be made before using them in the pots. The potting mixture comprised of topsoil, rough sand and organic matter in a ratio of 1:1:1 that gave a total porosity of 55%. Each sub treatment was replicated four times making a total number of 360 seeds for each main treatment making a total of 720 seeds for the whole trial. Moisture treatment levels applied per day in the first month (January 2010) were 100ml for low moisture level, 200ml for median level and 300ml for high level. During the subsequent two months moisture treatment was applied as 200ml for low level, 300ml for median level and 400ml for high level per day per pot. For the rest of months water was given as 400ml for low level, 800ml for median level and 1200ml for high level. Number of pots per treatment was 12pots, and 36pots made the subplots while the whole treatments comprised of 72 pots. Each pot carried 10 seeds. Sown seeds were inspected every day for germination and germination dates were recorded weekly for a period of 4 weeks per replicate. Seeds were considered germinated when the radical was about 2cm long and cotyledon had emerged from the seed coat. Data on germination percentage obtained were first transformed into arcsine transformation before computation but the original data was used in the summaries of the means [1]. Results from each experiment were analyzed separately. Means and Least Significant Difference (LSD) for germination percentages for both seed treatments and moisture levels were computed to get inference.

2.3 Rooting of the Cuttings

A split-split plot experimental design was used in both non-mist propagator and the open pots whereby populations from the three sites were assigned to the main plot, the potting media status (non inoculated and inoculated medium) was assigned to sub plots and cutting types were designated as sub-sub plots. Each main plot used soil from its respective site. Fresh soil from the sites was used without sterilization as inoculated media while media used as non-inoculated was sterilized in an oven at 120^0C for 24 hours. Sub samples of fresh and sterilized soil were kept for isolation and identification of mycorrhiza fungi in a separate experiment. Two non-mist propagators were used for stem and root cuttings respectively (Appendices 1 and 2). Treatment of cuttings was done according to Magingo et al. [7] and soil analysis of potting was done according to Allen [14]. Stem cuttings were inserted in the medium at a depth of 20mm while root cuttings were inserted in the medium at a depth of 15mm as standard procedures described by Leakey et al. [8]. Recordings were taken once a week and at every instance of opening the propagator, a thin spray of water was applied to maintain high and constant humidity. Parameters observed included sprouting, callus formation, rooting, weather conditions, seedling height, number of branches, root length, number of roots and characteristics of sampling areas (Tables 4, 5, 6). Weather was taken daily at 7.00 am, 12.00 noon and at 6.00 pm in the evening. A hygrothermometer RHT 20 was used for this purpose. Fungicide was occasionally sprayed inside the propagators to protect roots from rotting. Rooting was monitored for 3 months.

Cuttings set in the open space were raised in similar rooting media and watered daily to field capacity in a 20L pot per day for 90 days. Rooting experiments were conducted between January to March 2010 (wet season) and June to August 2010 (dry season).

3. RESULTS

3.1 *Dalbergia melanoxylon* seed Germination in MS Media

A total of 19.8% of seeds inoculated in half strength of MS medium germinated while only 6.8% of seeds inoculated in full strength of MS medium germinated (Tables 1, 2 and Plates 1, 2). This germination was at sterilization of 20 minutes in 35% ethanol and 20 minutes in 2.6% sodium hypochlorite at pH 5.8 and culture room condition of 28oC under a photoperiod of 16 hours at 3000 lux light intensity provided by cool white fluorescent tubes.

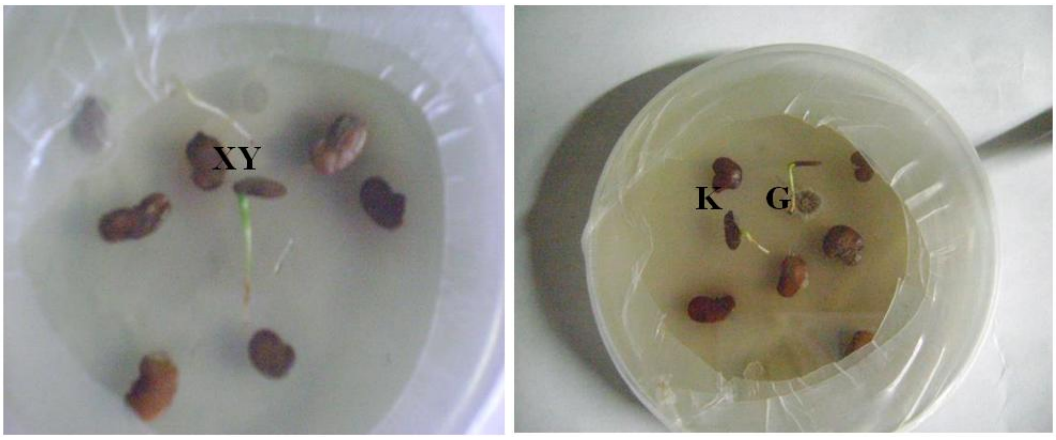
Table 1. *D. melanoxylon* seed germination in MS medium January-April 2010

Replica	#seed inoculated	#seed germinated	MS status	Sterilization time
1	50	0	1	1
2	50	3	1	1
3	50	2	1	3
4	50	5	2	3
5	50	3	2	3
6	50	0	1	3
7	50	10	2	2
8	50	2	2	2
9	50	0	1	1
10	50	0	1	3

NB: Full strength MS= 4.310 g/l, Half strength MS=2.155 g/l, MS stands for Murashige and Skoog (1962), MS status 1= Full strength, 2= Half strength, Sterilization status 1= 30 minutes, 2= 20 minutes, 3= 10 minutes

Table 2. One-sample statistics

	N	Mean germination	Std. Error Mean
Half Strength germination	10	10.40 ±2.716	.859
Full Strength germination	10	3.40 ±2.066	.653



Plates 1 and 2. Germinated seeds in MS media (Seed XY, G and K)

3.2 *Dalbergia melanoxylon* Seed Germination in the Soil

Germination percentage ranged from 2 to 21% in soaked seeds and 0.5 – 15% in non-soaked seeds starting from 7 days after sowing to 21days when all viable seeds had germinated. There was no statistically significant difference in seed germination percentage due to seed treatment. (Table 3 and plate 3). A maximum of 21% of seed germination in this experiment as indicated in Table 3 is relatively low. These results conform and agree with the results by Amri [12] in which germination was found to be between 20% to 50% while seed viability and seedling growth were also found to be relatively low.

Table 3. Main effect of seed treatment on seed germination

Seed treatment	Moisture level	Germination %				Mean	LSD
		January 2010	February 2010	March 2010	April 2010	Mean	
Soaking	Low (100 ml)	3.8	1.6	13.3	10.8	7.4	15..3
	Mediam (200 ml)	2.5	6.6	18.1	21.5	12.2	
	High (300 ml)	0.00	0.8	11.6	12.5	6.2	
	Mean Soaked	2.1	3.0	14.4	14.9	8.6	
Non soaking	Low (100 ml)	0.8	3.3	15.7	16.5	9.9	14.9
	Mediam (200 ml)	0.00	0.8	14.9	19.8	8.9	
	High (300 ml)	0.8	1.6	11.7	10.8	6.2	
	Mean Non soaked	0.5	1.9	14.1	15.7	8.4	
LSD	5%	2.99	4.43	5.61	3.65		
	1%	14.99	22.21	28.12	18.28		



Plate 3. Sample of germinated *D. melanoxylon* seed in the soil

3.3 Effect of Moisture Level on Seed Germination in the Soil

The effect of moisture level in the potting media on seed germination is as shown in Table 4. Generally germination increased from low to median moisture level. High moisture level was associated with low germination percentages at each date. The median moisture level was the most favorable in effecting seed germination. Here, germination ranged from 1.3 during the first week to 21% three weeks later when all viable seeds had germinated. This is opposed to 0.4 and 11.6% respectively for seeds received high moisture level. Percentage germination due to seed treatment was 15% and percentage germination due to moisture level was 21%. However the differences due to seed treatment and moisture level were not statistically significant. Other literature had reported that in–situ germination of *D. melanoxylon* seeds could decrease from 50% to 20% depending on the available factors affecting germination [4] including moisture status of the environment as was observed during conduction of this research.

Table 4. Mean seeds germination in response to different moisture level

Moisture level	January 2010	February 2010	March 2010	April 2010
Low	2.33	2.28	14.53	13.65
Median	1.25	3.73	16.55	20.68
High	0.41	1.24	11.67	11.64

3.4 Proportion of Rooting, Callusing and Sprouting under Different Treatments

The overall rooting ability was higher in the non-mist propagator than in the open nursery (Tables 5 & 6, Plate 4). At least 34% of the 400 cuttings set in the non mist propagator rooted while none rooted in the open nursery. This includes 100% of the softwood cuttings, 37% of the root cuttings and none of the semi-hardwood and hardwood cuttings set in the non-mist propagator rooted. About 54% of the 400 cuttings in the non mist propagator developed callus whereas only 38% of the 400 cuttings set in the open nursery did so. Likewise, 74% of cuttings in the non-mist propagator sprouted as opposed to only 38% in the open nursery (Tables 5 & 6, Plate 4). There was no significant difference in rooting percentage found between different populations which averaged 1.75%, 1.60% and 1.50% for Kilosa, Babati and Kilwa provenances respectively.

Table 5. Mean rooting characteristics in percentage recorded 3 months after the beginning of the experiment

	Rooting parameters					
Cutting type	Rotting %	Rotting %	Callus %	Callus %	Sprouting %	Sprouting %
	P	O	P	O	P	O
Softwood	100 ±1	00	50 ±4	40 ±3	50 ±9	40 ±3
Semi-hardwood	00	00	50 ±4	40 ±3	90 ±9	40 ±3
Hardwood	00	00	50 ±4	40 ±3	85 ±9	40 ±3
Root	37 ±1	00	67 ±4	30 ±3	70 ±9	30 ±3

(P=non-mist propagator, O= in the open pots)

However, significant differences in rooting percentage were found between cutting types and between potting media treatments. That is, 100% of softwood cuttings in the non-mist propagator and 37% root cuttings in the non-mist propagator rooted while none of the hardwood, semi-hardwood in the non mist propagator and the open nursery rooted (Table 5&6). There was a significant difference in sprouting percentage between cutting types and media treatments where 50% of softwood cuttings, 90% of semi-hardwood cuttings, 85% of hardwood cuttings as well as 70% of root cuttings in the non-mist propagator sprouted while only 40% softwood, semi-hardwood and hardwood cuttings and 30% of root cuttings in the open nursery sprouted. Callusing did not differ significantly since of softwood cuttings, semi-hardwood and hardwood cuttings in the non-mist propagator and 40% of all cutting types in the open nursery callused. About 67% of root cuttings in the non-mist propagator callused and only 30% root cuttings in the open nursery developed callus. Rooting, callusing, and sprouting percentage were relatively higher in cuttings placed in non-mist propagator compared to those placed in the open nursery.

Table 6. The proportion of rooting parameters between cutting types

Rooting Status				
Duncan [a,b]			**Subset**	
Age	**N**	**1**	**2**	**3**
Soft	100	1.0000		
Root	100		1.5657	
Semi	100			2.0000
Hard	100			2.0000
Significance	0.001		0.006	1.000

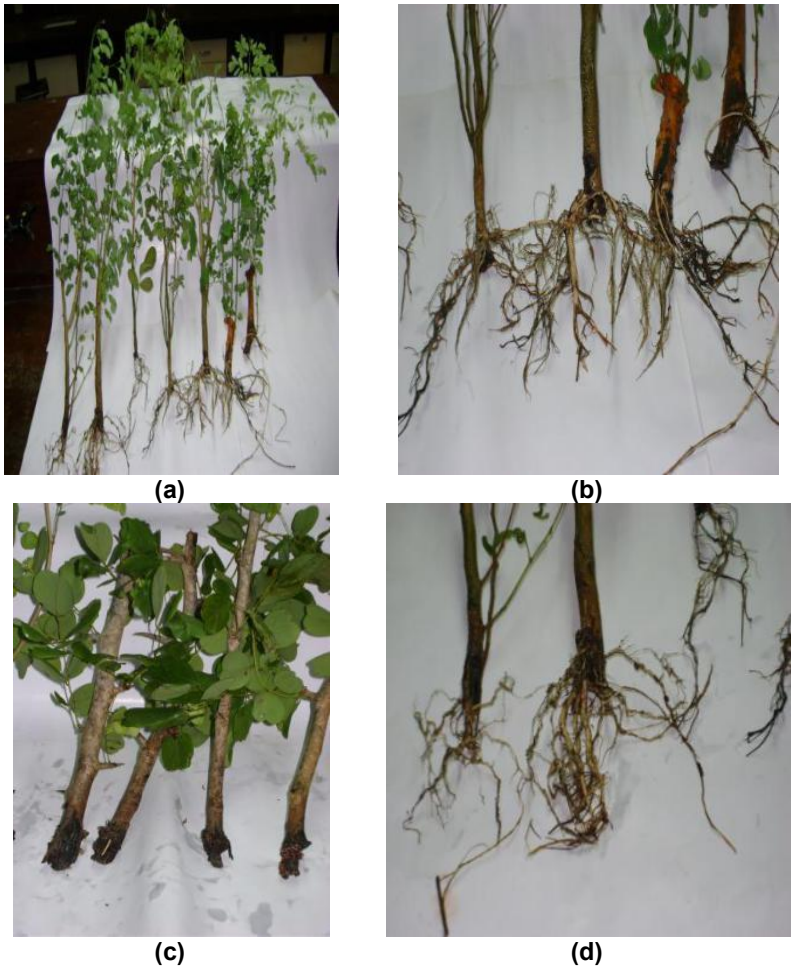


Plate 4. (a) Inoculated rooted softwood (b) Inoculated rooted root cuttings (c) Sprouted and callus formed inoculated and non-inoculated unrooted hardwood and semihardwood cuttings (d) Non-inoculated rooted root cuttings

4. DISCUSSION

When Murashige and Skoog medium is used for seed germination, callus production, shoot or root initiation what can affect the intended result is viability of the inoculated ex-plant (seed, root tip, shoot tip, leaf bud, embryo), concentration of the MS medium (Full strength or Half strength), concentration of the sterilizing reagents (e.g. 35% alcohol or 70% alcohol) and duration used to sterilize the ex-plants. If viability could be the only factor to determine germination in the MS in this experiment, it was

likely possible to get more than 50% germination as supported by viability test results. The co-factors are source of 19.8% germination in this experiment. For example when seeds were sterilized in 35% ethanol for 30 minutes and 2.6% sodium hypochlorite for 30 minutes, most of cultures remained uncontaminated but did not germinate. Three interpretation can be given to these results: one is successful sterilization but which killed the ex-plants, second is successful sterilization but there was no viable ex-plant in the trial, lastly is that some other necessary conditions was not attained such as light intensity or temperature. Other sterilization trials were subjected to 70% ethanol for 30 minutes and 2.6% sodium hypochlorite for 30 minutes. In this trial inoculums also did not get contaminated but also did not germinate the seed meaning that sterilization was successful but killed the ex- plants or sterilization was successful but there was no viable ex-plant in the trial or any other necessary condition was not attained. When only10 minutes was used to sterilize *D. melanoxylon,* ex-plants in either 35% ethanol and 2.6% hypochlorite or 70% ethanol and 2.6% sodium hypochlorite all inoculums gate contaminated meaning that this duration does not keep the ex-plants in aseptic conditions. When 20 minutes was used to sterilize *D. melanoxylon* ex-plants in either 35% ethanol and 2.6% hypochlorite or 70% ethanol and 2.6% sodium hypochlorite at least 19.8% of inoculated ex-plants germinated meaning that this duration did not kill cells or embryo of all ex-plants but also sterilized successful most of the viable ex-plants.

This range of concentrations and duration for sterilizing *D. melanoxylon* ex-plants was in agreement with Gamborg, (2002) who reported that “some plant species can respond to many media combinations, others can respond to only one media combination while others do not respond to any media combination”. It is evident from the study that seeds of *D. melanoxylon* are vulnerable to higher moisture level available which is in agreement with findings by Msanga, 1999 [9] . This was also pointed out by Arbonnier [3] that, *D. melanoxylon* seeds are orthodox (Moisture intorelant) needs 9-12% moisture content and 3oC to stay viable for a long period. Since seeds are indehiscent, papery, thin and flat, they easily get rotten when irrigated with high moisture. This implies that most seeds produced in natural environment yearly die during the rainy season. This is why 56% of the *in-situ* seedlings are reported to originate from root suckers [15]. Most domesticated plant species have been subjected to genetic transformation such as chromosomal and DNA recombination in such a way that they can be easily assessed and sterilized for tissue culture [16]. *D. melanoxylon* is one of the forest plant which is neither genetically transformed nor domesticated and therefore is colonized by various microorganisms hence is difficult to keep in aseptic conditions for tissue culture. For such forest plants it needs so many trials of sterilizing materials. Some treatment combinations of *D. melanoxylon* ex-plants in this study did not generate seed germination in the media because there were either no viable seed, sterilization was not enough or sterilization killed the seed embryos because the extent of sterilizing and ratios for the hormonal combination for *D. melanoxylon* was not known. For plant which is subjected to tissue culture for the first time such as *D. melanoxylon*, the conditions and parameters needs many trials to be known including hours of light exposure of inoculated materials, hours in darkness, quantity and quality of light intensity, temperature and type of ex-plant which can respond easily. All these have lead to difficulties for *D. melanoxylon* seed germination in the MS.

Based on Tables 5&6, there was significant difference at $P<0.05$ in rooting status, $P < 0.01$ in seedling height and $P < 0.05$ in root length between different media treatments. In other Tables indicated that these parameters were statistically not difference within the same media types and across the populations.

5. CONCLUSSION AND RECOMMENDATION

Dalbergia melaanoxylon low seed viability seems to be a strongest hindering factor of seedling production in both the soil and the MS medium since 21% germination in the soil and 19.8% of the MS does not show a statistically significant difference but soil germination is still have high percentage. Since the low seed viability have been reported from Redhead and Temu [6], Nshubemuki [4], Sharman [10], TTSA [11], Msanga [9], Amri [12] and Amri [13], two suggestions are given from this research, one is to genetically transform the species so that can be easily assessed to germinate and produce good number of seedlings, second is to use the few occurring seedlings from soil and MS germination in tissue culture for multiple production of seedlings through callus.

COMPETING INTERESTS

Author has declared that no competing interests exist.

REFERENCES

1. Zar JH. Biostatistical analysis 5th Edition, Prentice Hall Errata/ Corrections; 2010.IUCN. (2008). Red list of threatened plants. The IUCN species survival Commission, Royal Botanical Garden, Edinburgh.
2. IUCN. Red list of threatened plants. The IUCN species survival Commission. Royal Botanical Garden, Edinburgh; 2008.
3. Arbonnier M. Trees, shrubs and Lianas of West Africa dry zones. CIRAD, Montpellier: Meseum National d"historie Naturelle, Paris; 2004.
4. Nshubemuki L. Dalbergia melanoxylon: Valuable wood from a neglected tree. NFT Highlights 1993-1995. Nitrogen Fixing Tree Association, Hawaii; 1993.
5. Lamrood PY, Vaidya JG. Fungal genera of the Pune City. Department of Botany, Pune; 2001.
6. Redhead JF, Temu AB. Valued timber but neglected tree: Mpingo (Dalbergiamelanoxylon). Tanzania Association of Forester, Newsletter. 1981;2:8-9.
7. Magingo FSS, Dick JMcP. Propagation of two miombo trees by leafy stem cuttings obtained from seedlings. Centre for ecology and hydrology, Edinburgh University, India; 2001.
8. Leakey RRB, Tchoundejeu Z, Longman KA, Dick JMc,P Newton A, Martin A, Grace J, Munro RC, Mutoka PN. Low-technology techniques for vegetative propagation of tropical trees. Commonw. For. Rev. 1990;69:247-257.
9. Msanga HP. Different types of trees for plantations in different regions of Tanzania, MNRT Morogoro Tanzania; 1999.
10. Sharman H. Investigation in the sustainable management of tropical timber species using Dalbergia melanoxylon as a case study. Unpublished MSc thesis, University of Edinburgh. 1995;68-69.
11. TTSA. Handling of seeds of Dalbergia melanoxylon (African Blackwood). Seed issue note No. 9; 1995.
12. Amri E. Effect of timing of seed collections an provenances on seed viability and germination capacity of dalbergia melanoxylon. Bot Res. J. 2008;1(4):82-88.
13. Amri E. Effect of age of the donor plant, IBA treatment and cutting position to the rooting ability of stem cuttings in Dalbergia melanoxylon. New Foresters. 2010;38. DOI:10.1007/s11056-009-9163-6
14. Allen ES. Chemical analysis of ecological materials. Blackwell Scientific Publications, Massachusets; 1989.
15. Washa, BW. Dependence of dalbergia melanoxylon natural population on root Suckers regeneration. Journal of African Affairs. Hankuk University of Foreign studies, Seoul, Korea. 2008;24:177–198.
16. Kumar C, Singh AK, Nehar S. *In-vitro* propagation and callus formation from seedling of Terminalia arjuna Roxb. Bioscan. J. Life Sci. 2010;2:423-429.

Biography of author(s)



Dr. Washa B. Washa
University of Dar es Salaam – Mkwawa College (MUCE), P.O.Box. Private Bag, Iringa, Tanzania.

He was born in 1965 in Tanzania has three remarkable periods in his profession experience. One is a period of Teaching Biology and Chemistry in Secondary schools of Tanzania in which professionals and experts of various discipline were produced through his teaching. He taught about 7 secondary schools during this period from 1994-2007. Second is a Hardship period of BSc and MSc studies without a study leave through the Open University of Tanzania from 2000-2004 for BSc and 2005-2007 for MSc. His childhood life in forestry environments was a source for him to take an MSc in plant biology particularly the African Blackwood (Dalbergia melanoxylon). Third is his contribution to the science of Botany through investigations in Dalbergia melanoxylon from 2005 to date. Has been the first researcher in the world to test tissue culture in Dalbergia melanoxylon and the second researcher to write a Review of the Literature of this species in 2014. He has been a paper reviewer in various Journals including Tanzania Journal of Science (2012), International Journal of Plant and Soil sciences (2019), Recent Journal of Science and Technology (2020) and the Journal of Cell Biotechnology and molecular Biology (2020). Recently, he has contributed to the Book Publisher International (SCIENCEDOMAIN) by publishing a book chapter in the book named as 'Recent Research Trends in Biological Sciences' Vol. 4. 2020 pages (123 – 132) Chapter 14. He is a Senior Lecturer in Botany at Mkwawa University College of Education, Biological sciences Department in Plant physiology, plant Anatomy, Mycorrhizae and plant Tissue culture from 2008 To date.

© Copyright (2021): Author(s). The licensee is the publisher (Book Publisher International).

DISCLAIMER
This chapter is an extended version of the article published by the same author(s) in the following journal.
International Journal of Plant and Forestry Sciences, 1(1): 1 - 9, 2014.

Chapter 3
Print ISBN: 978-93-90516-93-3, eBook ISBN: 978-93-90516-94-0

Phytohormones Production in Microalgae and the Methods of Characterization

Cam Van T. Do[1,2,3], Thuan Dang Tran[4] and Quang Trung Nguyen[1,2*]

DOI: 10.9734/bpi/rrab/v5/7060D

ABSTRACT

Microalgae have been reported to produce endogenous phytohormones including auxins, gibberellins, cytokinins, brassinosteroids and abscisic acid. There is a vast increasing demand for microalgal-derived biomolecules in crop production (biostimulants and biofertilisers), food and feed, pharmaceutical, nutraceutical, and biofuel production. Biorefinery is a comprehensive approach that could fractionize all biocompositions of microalgal biomass for these specific uses. Methanol residual released from microalgal lipid extraction usually contains variety of bioactive compounds including the phytohormones have been reported to enhance crop seed germination and plant growth (root length, plant height, branch, leave and flower increase). Although some good results have been achieved on application of microalgal products as fertilizers, biostimulants, and regulators of plant growth, these studies poorly provided analytical techniques for characterization of these algal products. The profile and content of algal products-constituted biomolecules which play the main roles in catalyzing crop seed germination as well as plant growth should be elucidated for providing basic understanding and guiding for specific applications in agriculture. This book chapter is aimed at representing production of various phytohormones production in microalgae, cyanobacteria, as well as algae world. Moreover, this study also provides state of the art of recent advanced techniques in qualitatively and quantitatively determination of phytohormones in microalgae. Moreover, general perspective of algal phytohormones applications in the regard of microalgae-based biorefinery is also overviewed.

Keywords: Microalgae; phytohormones; analytical techniques; biorefinery.

1. INTRODUCTION

1.1 Phytohormones

Phytohormones or growth hormones are small organic molecules or substances produced naturally by plants at very low concentrations that activates or regulates their growth such as physiological processes in plants [1]. Phytohormones are presented as chemical messengers that coordinate cellular activities (division, elongation and differentiation) of plants, parttern formation, organogenesis, reproduction, sex determination and responses to abiotic and biotic stress [1]. Currently, ten groups of phytohormones have been discovered and identified including auxins (first discovered in 1935) [2], cytokinins (CK) [3], gibberellins (GA) [4], abscisic acid (ABA) [5], ethylene (ET) [6], brassinosteroids (BR) [7], salicylates (SA) [8], jasmonates (JA) [9], strigolactones (SL) [10] and peptide hormones [11]. The first five (auxin, CK, GA, ABA, and ET) are generally ranked as the “classical” phytohormones, while the latter four are emerging phytohormonal family [12]. Structurally, auxins are indole derivatives; ABA is a sesquiterpene; ETH is the simplest alkene; CKs are adenine derivatives; GAs are tetracyclic diterpenoid acids; BRs are polyhydroxysteroids; JA is a fatty acid derivative from linolenic acid; SLs are terpenoid lactones derived from carotenoids (Fig. 1) [13-17]. Basically, auxins,

[1]Graduate University of Science and Technology, Vietnam Academy of Science and Technology, 18 Hoang Quoc Viet, Cau Giay, Ha Noi, Vietnam.
[2]Center for Research and Technology Transfer, Vietnam Academy of Science and Technology, 18 Hoang Quoc Viet, Cau Giay, Ha Noi, Vietnam.
[3]Department of Chemical Technology, Hanoi University of Industry, No. 298 Cau Dien, Bac Tu Liem, Ha Noi, Vietnam.
[4]Institute of Chemistry, Vietnam Academy of Science and Technology, 18 Hoang Quoc Viet, Cau Giay, Ha Noi, Vietnam.
**Corresponding author: E-mail: nqt79@yahoo.com;*

GAs, ABA, JA, and SA are acidic and CKs are alkaline; thus, the diversity of chemical structures and properties of different phytohormones make the specialized extraction, purification, and quantification, as well as localization, procedures necessary (Fig. 1) [13-17]. It has been suggested that higher plant hormone systems evolved from a pre-existing primary metabolic system in microalgae [18], however, evidence for the existence and function of phytohormones in microalgae still remains unknown [19].

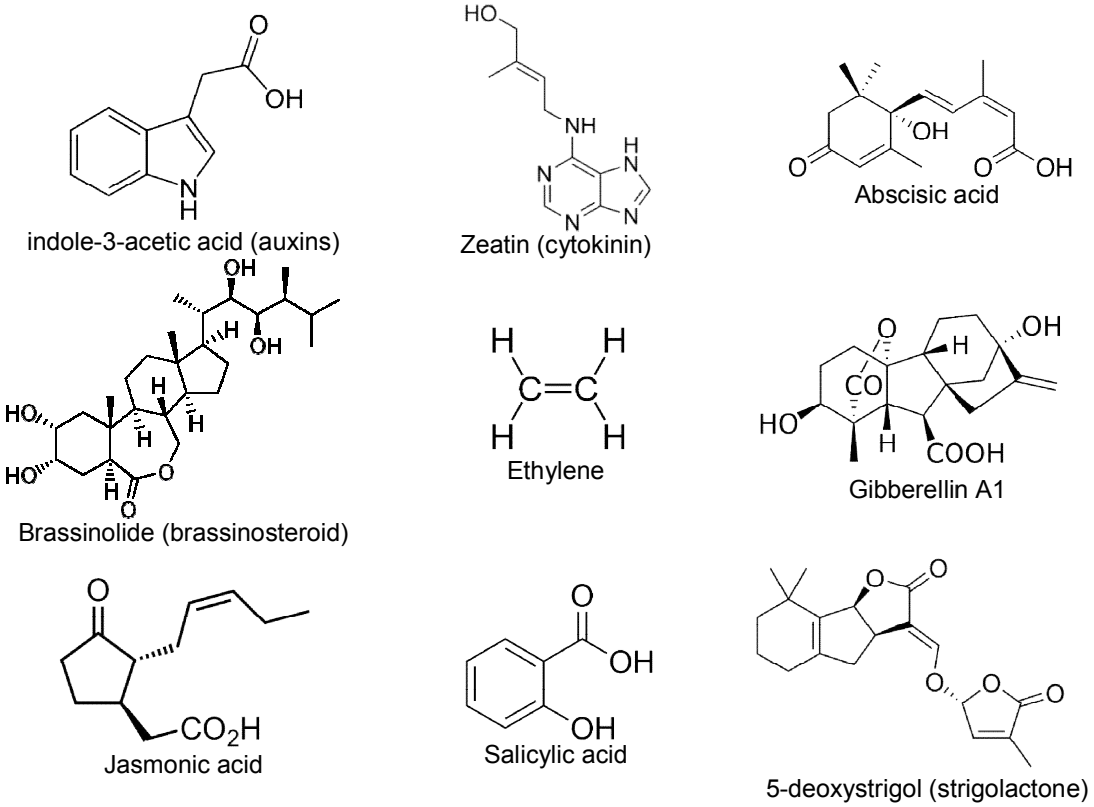


Fig. 1. Chemical structure of classical and emerging phytohormones [2-11]

1.2 Phytohormone Production by Algae and Cyanobacteria

1.2.1 Phytohormones producing strains

It has been reported that microalgae could produce exogenous [20] and endogenous phytohormones [21-26]. The most common phytohormones reported to produce in microalgae were auxins [24], gibberellins [22], cytokinins [24], brassinosteroids [21,22], and abscisic acid [27]. The production capacity of phytohormones in green algae such as *Chlorella* sp. [21,26], *Scenedesmus* sp. [20, 28], *Nannochloropsis oceanica* [29] and *Acutodesmus dimorphus* [30] have been reported recently (Table 1). Moreover, cyanobacteria were also reported to produce various phytohormones including auxins (i.e., *Synechocystis* sp., *Chroococcidiopsis* sp., *Anabaena* sp., *Phormidium* sp., *Oscillatoria* sp., *Nostoc* sp.) [31-33], ethylene (i.e., *Synechococcus* sp., *Anabaena* sp., *Nostoc* sp., *Calothrix* sp., *Scytonema* sp. and *Cylindrospermum* sp.) [34], abscisic acid (i.e., *Synechococcus leopoliensis*, *Nostoc muscorum*, *Trichormus variabilis* and *Anabaena variabilis*) [27,35,36], cytokinin (i.e., *Synechocystis* sp., *Chroococcidiopsis* sp., *Anabaena* sp., *Phormidium* sp., *Oscillatoria* sp., *Calothrix* sp., *Chlorogloeopsis* sp. and *Rhodospirillum* sp.) [32,37] and gibberellins (i.e., *Anabaenopsis* sp., *Cylindrospermum* sp., *Phormidium foveolarum*) [37,38] (Table 1). Diatoms (i.e., *Ecklonia* sp.), eustigmatophytes (i.e., *Nannochloropsis oceanica*), brown algae (i.e., *Ectocarpus siliculosus*, *Padina arborescens*, *Ecklonia maxima*, etc.), red algae (*Prionitis lanceolata*, *Porphyra* sp., *Gelidium* sp., *Gracilaria* sp., *Gracilariopsis* sp., *Chondracanthus* sp., *Hypne*a sp., etc.) were also identified to produced various phytohormones (Table 1).

Table 1. Cyanobacteria and microalgae producing phytohormones

Cyanobacteria/microalgae	Phytohormones					Reference
	Auxin	**ET**	**ABA**	**CK**	**GA**	
Synechocystis sp., *Chroococcidiopsis* sp., *Anabaena* sp., *Phormidium* sp., *Oscillatoria* sp., *Nostoc* sp. *Ectocarpus siliculosus* *Prionitis lanceolata*, *Porphyra* sp., *Gelidium* sp., *Gracilaria* sp., *Gracilariopsis* sp., *Chondracanthus* sp., *Hypnea* sp. *Scenedesmus armatus*, *Chlorella pyrenoidosa*, *Chlorella inutissima*	Yes	-	-	-	-	[21, 26, 31-33, 39-43]
Synechococcus sp., *Anabaena* sp., *Nostoc* sp., *Calothrix* sp., *Scytonema* sp., *Cylindrospermum* sp., *Padina arborescens*, *Ecklonia maxima,* *Porphyra tenera,* *Chlorella pyrenoidosa*	-	Yes	-	-	-	[34, 44, 45]
Synechococcus leopoliensis, *Nostoc muscorum*, *Trichormus variabilis*, *Anabaena variabilis* *Nannochloropsis oceanica* *Ascophyllum nodosum* *Porphyra* sp., *Gelidium* sp., *Gracilaria* sp., *Gracilariopsis* sp., *Chondracanthus* sp., *Hypnea* sp. *Chlamydomonas reinhardtii*, *Dunaliella* sp., *Draparnaldia mutabilis*, *Chlorella minutissima*	-	-	Yes	-	-	[27, 35, 36, 43, 46, 47]
Synechocystis sp., *Chroococcidiopsis* sp., *Anabaena* sp., *Phormidium* sp., *Oscillatoria* sp., *Calothrix* sp., *Chlorogloeopsis* sp., *Rhodospirillum* sp., *Ecklonia* sp., *Nannochloropsis oceanica,* *Ecklonia maxima*, *Laminaria pallida,* *Porphyra* sp., *Gelidium* sp., *Gracilaria* sp., *Gracilariopsis* sp., *Chondracanthus* sp.,	-	-	-	Yes	-	[29, 32, 37, 48-50]

Cyanobacteria/microalgae	Phytohormones					Reference
	Auxin	ET	ABA	CK	GA	
Hypnea sp., *Gigartina clathrata*, *Hypnea* sp. *Chlorella minutissima*						
Anabaenopsis sp., *Cylindrospermum* sp., *Phormidium foveolarum* *Nannochloropsis oceanica* *Ecklonia radiata* *Hypnea musciformis,* *Chlorella* sp., *Chlamydomonas reinhardtii*	-	-	-	-	Yes	[22, 38, 51, 52]

Auxin; abscisic acid: ABA; cytokinin: CK; ethylene: ET; gibberellins: Gas

1.2.2 Factor affecting phytohormones production by microalgae

1.2.2.1 Effect of light on production phytohormones by microalgae

Photosynthesis of microalgae to growth biomass is driven by light as energy source. It was demonstrated that algal cell division, light and cytokinins production are inter-related [26]. Stirk et al. [26] used synchronized *Chlorella minutissima* Fott et Nova´kova (MACC 361) and *Chlorella* sp. (MACC 458) to study effect of light on cytokinin concentrations and cell cycle by growing in a 14:10 light:dark (L:D) photoperiod. It was revealed that cell division occurred during the dark period and cells increased in size during the light period. Notebly, cytokinin profiles including five *cis*-zeatin (cZ) and three N6-(2-isopentenyl)adenine (iP) derivatives were similar in both strains and their concentration were low during the dark period and increased during the light period. Moreover, cytokinins were detected in all samples with concentrations increased over the first 24 h of the cultivation. Particularly, the large increase (4 fold) was observed when these two microalgal species experiencing the second light period. However, cytokinin concentrations measured for the two algal species did not increase when the cultures maintained in continuous dark conditions [26].

In another study, the effect of light on growth and endogenous plant growth regulators (PGRs) concentrations in microalgae was investigated by Stirk et al. [21]. *Chlorella minutissima* MACC 360 was grown in 14:10 h light:dark (L:D), continuous dark (CD) and continuous dark with the addition of 5 g/L glucose (CD + G) for 48 h. Cultures were synchronized in the L:D cultures, increasing in size during the light period and dividing during the dark period. *C. minutissima* cells did not increase in size or undergo cell division in CD cultures. In CD + G conditions, the cultures were no longer synchronized but did continue to increase in cell size and constantly underwent cell division although fewer cells divided than in the L:D cultures. Endogenous auxin and cytokinin concentrations increased and gibberellin concentrations decreased over time in the actively growing cultures (L:D and CD + G) but did not increase in the CD cultures. The largest increase in indole content was in the CD + G cultures while the L:D cultures had the largest cytokinin increase. Brassinosteroid concentrations decreased over time in all the cultures including those grown in CD conditions. Abscisic acid (ABA) concentrations were low and only increased in the CD cultures. These results show that endogenous PGRs were affected by the light regime and/or culture growth [21].

1.2.2.2 Effect of stress on growth, profile and levels of auxins, cytokinins, and gibberellins produced by microalgae

There are two engineering strategies that were often used to enhance lipids production in microalgae reported in the literature. These are growing microalgae in high salt concentration and/or nitrogen-deficient conditions. It was commonly reported that increasing NaCl concentration in culture media improves algal lipid content but decreases the algal growth (e.g., *Scenedesmus obliquus*, *Chlorella vulgaris*) [53, 54]. In consistence with the reported data, the *C. sorokiniana* TH01 used in our study was also exhibited specific growth rate and biomass concentration decreasing from 0.72 to 0.23 1/day and 0.96 to 0.55 g/L, respectively, when NaCl concentration increased from 0 to 20 g/L. Notably, the lipid content of *C. sorokiniana* TH01 derived in the form of fatty acid methyl esters (FAME) were

determined as 19.13, 24.34, 33.48, 39.75 and 44.23% DCW when 0, 5, 10, 15 and 20 g/L NaCl were introduced as a medium composition [55].

For phytohormones production, it was reported that the IAA, IPA and GA7 contents determined for *C. sorokiniana* TH01 grown in BG-11 media under autotrophic without salt stress were 0.93, 2.66 and 20.87 µg/g dry weight (DW), respectively. The contents of IAA, IPA and GA7 were observed to decrease to 0.49, 2.55 and 13.05 µg/g DW, respectively, when NaCl was supplied to BG-11 medium at concentration of 5 g/L. Levels of IAA, IPA and GA7 were continued decreasing to 0.44, 1.98 and 8.71 µg/g DW, 0.36, 1.02 and 6.8 µg/g DW and 0.18, 0.8 and 3.39 µg/g DW, respectively, when NaCl concentration accordingly increased to 10, 15 and 20 g/L, respectively. Total auxins was measured to decrease from 3.59 to 0.98 µg/g DW when NaCl concentration increased from 0 to 20 g/L, respectively. The reduction of auxins levels measured for *C. sorokiniana* TH01 under salt-stress conditions is agreement with observation of Du et al. [56], who reported 72% decrease of endogenous IAA content in rice after experiencing 3 days of drought stress. However, in another study, growth of rice in a cold condition for 3 days and a heat stress for 6 h resulted in 1.6-fold and 1.3-fold increases of IAA levels [57], respectively.

Contrastingly, GA4 content was observed to steadily increase from 2.84 µg/g DW to 6.26 µg/g DW when NaCl concentration increased from 0 to 20 g/L. However, total GAs content decreased from 23.71 µg/g DW to 9.65 µg/g DW when NaCl concentration increased from 0 to 20 g/L. Consistently, reduction of GA levels and signalling has been shown to contribute to plant growth restriction on exposure to several stresses, including cold, salt and osmotic stress [58].

External addition of various phytohormones to culture media have been reported to stimulate the growth and stress tolerance of various microalgae [59,60]. However, characterization of endogenous phytohormone levels in microalgae under stress conditions are very limited [29,30]. Nitrogen starvation (nitrogen stress) have been reported to decrease abscisic acid (ABA) and IAA levels in *Acutodesmus dimorphus* from 5.07 and 556.83 pM/g fresh weight (FW) (control) to 4.7 and 376.40 pM/g FW, respectively, in the first day of N-starvation. However, ABA and IAA levels were observed to increase to 5.82 and 585.08 pM/g FW, respectively, when *Acutodesmus dimorphus* experienced the third day of N-starvation. [30] Study conducted by Lu et al. [29] also reported that transcriptional up-regulation of the biosynthetic pathways of ABA was occurred in *N. oceanica* under the N-deficient. Moreover, results achieved by Sulochana and Arumugam [61] documented that an immediate increase of ABA levels to up to four folds over the control in *S. quadricauda* during the onset of 24 h of nitrogen deprivation; however this ABA trend was reversal when N-starvation was continued up to 72 h. Similarity, ABA was reported to increase its level in *Dunaliella parva* [62] and *Draparnaldia mutabilis* [27] when they were grown in conditions of salt stress and increased pH.

1.2.2.3 Effect of carbon source on growth, profile and levels of auxins, cytokinins, and gibberellins produced by C. sorokiniana TH01 under mixtrophic growth

Mixtrophic growth of *Chlorella sorokiniana* using both sunlight and organic carbon as energy sources has been reported to achieve higher growth rate, biomass productivity [63,64] and lipid content [65]. Therefore, the cultivation mode is also a promising strategy for achieving two targets of both biomass and lipid production for biofuels application. It was reported that the significant increase of specific growth rate, biomass concentration and lipid content were simultaneously observed when glucose added as organic carbon source in BG-11 medium for mixtrophic growth of *C. sorokiniana* TH01. The specific growth rate, biomass concentration and lipid content measured for *C. sorokiniana* TH01 were 3.34, 3.63 and 3.16 1/day; 2.45, 4.41 and 5.46 g/L and 21.38, 29.56 and 37.36% DCW when glucose concentration added at 2, 4 and 6 g/L, respectively. Our obtained data was slightly better than data (specific growth rate of 3.20 – 3.40 1/day, biomass concentration of 1.66 – 5.08 g/L, lipid of 13.4 – 34.7% DCW) reported for *C. sorokiniana* (UTEX 1602) grown mixtrophically in Kuhl medium supplemented with glucose concentration of 2 – 20 g/L [65].

Since metabolism of microalgae varies with variation of culture conditions. That results in changing of hormones profile as well as their concentrations. Do et al. [55] that endogenous IAA, IPA and GA4 levels produced by *C. sorokiniana* TH01 were observed to increase from 0.93, 2.66 and 3.84 µg/g DW and peaked at 1.83, 3.25 and 7.88 µg/g DW when *C. sorokiniana* TH01 was turned from phototrophic mode (no sugar added) to mixtrophic mode with 2 g/L glucose supplement. However, the decreased

trend of IAA and GA4 contents to 0.74 and 2.98 µg/g DW were recorded when glucose concentration increased to 4 g/L. The IPA content was represented to be disappeared at glucose concentration added of 4 and 6 g/L. GA4 content was also disappeared at glucose concentration used of 6 g/L, while IAA was measured at 0.56 µg/g DW. The variation trend of endogenous GA7 of *C. sorokiniana* TH01 grown mixtrophically was decreased from 20.87 to 2.85 µg/g DW as glucose concentration increased from 0 to 6 g/L.

Among 15 phytohormones belonged to three groups of auxins, cytokinins and gibberellins, *C. sorokiniana* TH01 was detected to produce auxins and gibberellins. The main auxins detected in *C. sorokiniana* TH01 was IAA with lower level than IPA. The results were agreement with observation of Le Bail et al. [39] who also detected IAA, ICA and IPA co-produced in *Ectocarpus siliculosus* (Phaeophyceae). Contrastingly, study of Stirk et al. [24] reported to detect 0.50 – 71.49 nmol IAA/g DW and 0.18 – 99.83 nmol indole-3-acetamide (IAM)/g DW in 19 microalgal strains (including Chlorophyceae, Trebouxiophyceae, Ulvophyceae and Charophyceae) grown phototrophically and harvested at the exponential stage. Similarity, Jirásková *et al*. [66] also reported a profile of auxins produced by seven *Chlorella* strain harvested at the stationary phase including IAA at higher content than IAM.

The gibberellins profile produced by *C. sorokiniana* TH01 was characterized as GA4 and GA7. In the recent literature, gibberellins were all detected in the all 24 microgal strains (including Chlorophyceae, Trebouxiophyceae, Ulvophyceae and Charophyceae) with concentration ranging from 342.7 ng/g DW in *Raphidocelis subcapitata* MACC and 317 – 4746.1 ng/g in *Scotiellopsis terrestris* MACC 44 [22]. More interestingly, Stirk et al. [21] carried out a study of effect of light on growth and endogenous hormones in *Chlorella minutissima* indicated that plant growth regulators (PGRs) including auxins, cytokinins and gibberellins (GAs) were all detected in the *Chlorella minutissima* biomass under investigated conditions. The concentration of auxins, cytokinins and gibberellins (GAs) were determined as 0.85 – 26.8 µg/g, 0.15 – 0.80 µg/g and 0.94 – 5.29 µg/g, respectively. The limited number of phytohormoes detected in *C. sorokiniana* TH01 when compared to that of other strains reported in the literature is because profile of hormones produced by microalgae depending on the nature of species, growth conditions, nutrient conditions as well as extraction and analytical techniques.

1.2.2.4 Growth, profile and endogenous auxins, cytokinins and gibberellins levels of C. sorokiniana TH01 grown phototrophically outdoor

Although outdoor cultivation of microalgae in raceway ponds hardly controls algal growth and targeted products production, the technology is considered as effective and inexpensive methods for commercial production of algal biomass. In an our study, a raceway pond of 3.785 m^2 [67] was used for pilot production of *C. sorokiniana* TH01 in BG-11 medium under outdoor conditions. The reported data illustrates that the hormone profiles characterized for *C. sorokiniana* TH01 grown outdoor were IAA, IPA, GA4 and GA7 with concentrations in the crude water extracts expressed as 124.6, 938.1, 75.3 and 1924.8 µg/L, which were equivalent to phytohormones levels in the algal biomass of 0.98, 7.40, 0.59 and 15.18 µg/g, respectively. The total auxins and gibberellins in the water extract were 1062.7 and 2000.1 µg/L, respectively. The results further pointed out that levels of auxins and gibberellins produced by *C. sorokiniana* TH01 in phototrophic cultivation under outdoor conditions were notably lower than indoor conditions in both phototrophic and mixtrophic (with glucose concentration < 2 g/L) growths. The reasons of these achieved data were still unknown [55].

2. PRODUCTION OF MICROALGAL BIOMASS

Production of microalgal biomass can be performed in either phototrophic, heterotrophic and mixtrophic cultivation modes [64, 65]. Phototrophic production of microalgae requires inorganic carbons such as carbon dioxide or bicarbonate and light as an energy source for the algae growth [68]. Several microalgae (i.e., *Chlorella*) can be grown heterotrophically by using organic carbon such as glucose, acetate, glycerol, etc. as chemical an energy source to build up biomass constituents [69-72]. In mixtrophic cultivation mode, microalgae simultaneously consume organic and inorganic carbons using light as energy source to build up biomass [65,73]. Regarding to reactor technology, production of microalgae can be implement in either open and/or closed reactor system [74,75].

Closed reactors can be in the form of tubular, column, flat-panels and bag photobioreactors. Open reactor system is usually employed designs such as open raceway and thin-layer [76,77]. Although numerous reactor systems have been designed for research and pilot-production of microalgae, only raceway and thin-layer reactor are economically feasible for production of microalgae at commercial scale [67,78-80]. For heterotrophic microalgae, the production can be performed by fermentation reactors under completely sterilized medium [81]. However, the most sustainable microalgae production route is cultivation in wastewater for simultaneously removal of nutrients (carbon, nitrogen, phosphorous) and biomass production due to large saving of chemical fertilizers as well as water consumption [82].

3. METHOD OF CHARACTERIZATION AND DETERMINATION MICROLAGAL PHYTOHORMONE

Development of a method for simultaneous quantification of multiple classes of plant hormones is critical important as it can provide a basis for understanding the phytohormone profiles produced by microalgae. In addition, in order to study the functions of plant hormones, it is necessary to determine the concentration of endogenous plant hormone, while determination of exogenous plant hormone concentration would guide us to apply algal suspension for agriculture as foliar fertilizer for plant growth promotion [83]. Phytohormones usually occurs and acts in microalgae at very low concentration (trace amounts) with many similar structures, thus the determination of phytohormone concentration in microalgae is extremely challenging. Therefore, the analytical method used for microalgal hormones requires an efficient extraction procedures and high sensitive and selective analytical systems.

Numerous methods have been used for analysis of endogenous phytohormones in microalgal biomass. The conventional tests are phytohormones-like activity bioassays [25] and enzyme-linked immunosorbent assay (ELISA) [27] for quantitative determination of plant hormone due to their specificity, sensitivity and relative low cost. However, quantification using immunoassay can be misleading because of cross-reactivity of antibodies or inhibition with interfering substances. Additionally, complex preparation of antibody and the low purity of the plant extracts also can hamper the immunoassay analysis. High-performance liquid chromatography coupled with UV-VIS detector (HPLC/UV-Vis) allows to obtain phytohormone concentration faster with more precise, but only indole-3-acetic acid (IAA) was detected [20]. This indicates that the sensitivity and specificity of HPLC with electrochemical detection owns a inevitable limit for the measurement of various endogenous phytohormones at different levels in biomass samples. Fortunately, high performance liquid chromatography coupled with mass spectrometer detector (HPLC-MS) making an advanced technique which has been used for successfully structure elucidation and quantification of multiclass of phytohormones in a variety of biomass matrixes such as macroalgae [23], plants [84], seeds[85] and microalgal biomass [21,22,24,26,28].

The use of mass spectrometer as a detector for structure elucidation and quantification of phytohormones as a high sensitivity and selectivity technique have been applied in a variety of biomass matrixes such as macroalgae [23,49,86,87], plants [84,88-91], seeds [85,92], liquid biofertilisers [93]. However, there is a few study utilizing MS for profiling and quantitatively determination of endogenous phytohormones in microalgal biomass [21,22,24,26,28]. Moreover, efforts have been made on application of advanced analytical techniques such as solid-state ^{13}C Magic Angle Spinning Nuclear Magnetic Resonance (MAS NMR), Fourier-transform Infrared Spectroscopy (FT-IR), Inductively Coupled Plasma Atomic Emission Spectroscopy (ICP-EOS) and Elementar vario MACRO CNS for characterization to understand insight the complexity and heterogeneity of various bioactive components of methanol extracts of *Chlorella vulgaris* and *Scenedesmus quadricauda* [94].

4. APPLICATION OF ALGAL PHYTOHORMONES

There is a considerable growing demand for microalgal products in crop production (biostimulants and biofertilisers) [95], food and feed [96], pharmaceutical [97], nutraceutical [98] and biofuel production [99]. Therefore, much researches are currently focused on improving microalgal productivity [100-102]. One of potential application of microalgal hormones is to catalyze algae growth [103,104].

Indeed, understanding the roles that plant hormones play in the regulation of microalgal growth will potentially enable cultures to be manipulated to improve growth and yields. In crop research, numerous studied have been carried out on tomato to develop microalgae-based bio-stimulants, which can enhance seed germination, lateral and longitudinal root formation, roots nutrient uptake, improving total volume and vigor of the root system, increasing number of flower and braches. In a recent study, tomato seed treated with higher than 50% (v/v) of *A. dimorphus* extract concentrations exhibited an enhanced growth [105]. The improvement rate of germination (10 – 50%) of maize and wheat seeds was observed when these seeds treated with *Chlorella vulgaris* culture harvested from both phototrophic and heterotrophic growths compared to the control experiments [106]. Moreover, germination of tomato and cucumber seeds treated with 0.17 and 0.25 g/L of *Chlorella vulgaris* suspension grown phototrophically was enhanced when compared to those of the control (sterilized culture medium) [107]. In another study, the stimulating effect of *C. vulgaris* was observed on tomato plant while inhibiting the growing of *Meloidogyne arenaria* nematode parasite [108]. It was also known in study of Oancea et al. [109]. that the use of *Nannochloris* sp. 424-1 derived biostimulant favored the growth of non-water stressed tomato plants. Furthermore, some of marine algal extracts were tested on tomato seedlings with pretty improvement in germination rate and plant growth. More recent study of Abd Elhafiz et al. [110] pointed out that salt tolerant *C. pyrenoidosa* culture improved the germination rate of cucumber and root system. The filtrate of *Chlorella sorokiniana* was also found to increase *Triticum aestivum*'s lengh, total biomass aboveground and belowground by 30, 22 and 51%, respectively [111]. Growth and qualities of Chinese chives and spinach in organic farm was notably improved when the plants treated with 0.4% *Chlorella fusca* culture (1.5×10^7 cells/mL) and decreased the gray model disease [112]. Treatment of *Beta vulgaris* L. seedlings with water extract of *Chlorella vulgaris* and *Scenedesmus quadricauda* significantly improved root traits related to soil exploration and nutrient uptake such as root length, fine root length and number of root tips than the control [94]. Moreover, the *Scenedesmus quadricauda* extract positively affected the growth of lettuce seedlings, presenting an increase in dry matter, chlorophylls, carotenoids, proteins, and influencing the activities of several enzymes involved in the primary metabolism [113].

The enhancement of seed germination, plant growth and quantity are attributed to the microalgal culture and algal extract contain phytohormones such as auxins and giberrellins which are characterized in this study and cytokinins and kinetins reported in Oancea et al. [109]. Moreover, the microalgal extracts also contained various active compounds such as proteins, carbohydrate, vitamin as well as elemental compositions [114]. Recent studies have demonstrated that auxin controls seed dormancy [115] and exogenous auxin treatment represses plant seeds (e.g., soybean) germination by enhancing ABA biosynthesis [116] whereas treatment with exogenous GA (e.g., GA3) improved seed germination and seedling of Masson pine (*Pinus massoniana* Lamb.) [117]. Our recently reported data indicated that Millipore water extract of *C. sorokiniana* TH01 contains both auxins and gibberellins with total concentration of 1062.7 and 2000.1 µg/L, respectively, representing gibberellins concentration is almost 2-fold higher than that of auxins. Thus, the enhancement of tomato and rice seed germination was attributed to the promoting role of gibberellins in the crude algal extract. Moreover, the active concentration of auxins and gibberellins for triggering earlier germination of seeds of rice and tomato plants were determined as 425.08 and 800.04 µg/L, respectively.

5. UTILIZATION OF ALGAL PHYTOHORMONES IN BIO-REFINERY PERSPECTIVE

The use of algal biomass-filtered media for crop production is absolutely interesting due to the method can simultaneously utilize exogenous algal phytohormones and residual nutrients in algal culture medium for stimulating plant growth. However, the utilization of whole algal biomass for the crop production purpose only might be wasteful. This is because microalgal biomass is considered as a high value biomaterial which usually contains large content of lipids and/or carbohydrates and proteins [67]. In the regard of biorefinery, microalgal lipids and carbohydrates are promising feedstocks for liquid biofuels (biodiesel, bioethanol, etc.) production [118] whereas proteins [119] are potential materials for feed/food formulation [120]. On the other hand, lipid and sugar extractions are pre-requisite steps in biofuels production from algal biomass. The extraction of lipids is usually performed with biphasic solvents of chloroform/methanol or hexane/methanol to obtain lipids dissolved in less polar solvents (e.g., chloroform, hexane) [121], while the methanol phase contains pigments and variety of bioactive compounds (phytohormones, anti-bacteria biomolecules, anti-

cancer biomolecules, etc.) [122]. Currently, there are only few studies reporting to use the methanol extracts derived from *Chlorella vulgaris* and *Scenedesmus quadricauda* to study biostimulating effects on lettuce seedlings [113], morphological and molecular responses in *Beta vulgaris* L. [94] and biological activity on soil [123]. It has been reported that that endogenous algal phytohormones released out from algal biomass will migrate to methanol phase during lipid extraction. Therefore, collection of methanol phase for concentrating phytohormones would be advantageous for agricultural research. However, possibility of the use of the methanol extract for crop application is depended on profile and quantity of the phytohormones contained in the extract. Therefore, profiling and quantification of the phytohoromones in the methanol algal extract is necessarily as it provides a basic understanding of the extract toward reasonable applications in crop research. Although much researches are needed to develop a comprehensive microalgae-based biorefinery, it was pointed out that lipids of microalgae recovered from solvent extraction could be used for biofuels production and residual biomass could be used for feed/food formulation and/or biocomposite fabrication [124]. The remaining methanol residual can be used to recover methanol and obtain a concentrated material for agricultural research. This approach is a zero-waste refinery from microalgae feedstock (Fig. 2).

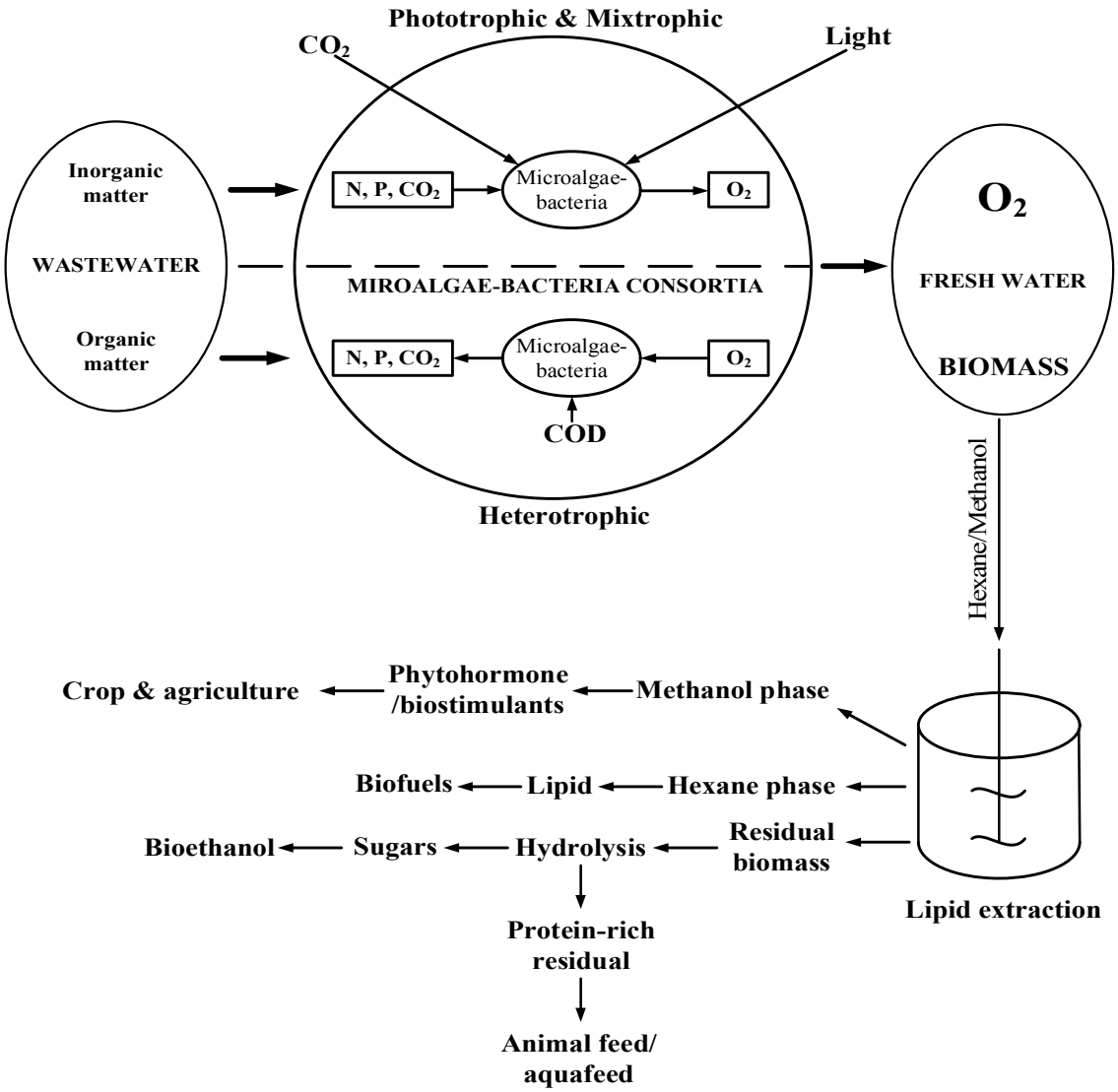


Fig. 2. Microalgae-based biorefinery

6. CONCLUSION

Microalgae-based biorefinery research is expanding as a consequence for growing demand in algae-based products in various industries (i.e., biofuels, food, feed and agriculture). Algal phytohormones become more important in the research of microalgae biotechnology itself as well as in the relevant fields (i.e., agriculture). A successful application of the algal phytohormones in the agriculture is requisitely decided by the economical production of microalgae at large and/or commercial scale. In this regards, cultivation of microalgae using the novel species in wastewater as a nutrient sole is an only sustainable culture approach to meet requirements of large volume and low price fields. Moreover, due to low concentration, the algal hormones needs to be processed and extracted from body of the algal biomass before employed for the targeted crops as well as researches. Therefore, these phytohormones need to be characterized with reliably analytical techniques. High-performance liquid chromatography coupled with mass spectrometry (HPLC-MS) is an adequate tool for profiling as well as quantification of endogenous and exogenous phytohormones produced by microalgae.

ACKNOWLEDGEMENTS

This research is funded by Graduate University of Science and Technology – Vietnam Academy of Science and Technology (GUST – VAST) under the grant number GUST.STS.ĐT2017-MT01.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Williams ME. Introduction to Phytohormones. Teaching tools in plant biology: lecture notes. , The Plant Cell. 2011;22.
2. Jiang Z, Li J, Qu LJ, 2 - Auxins, in: Li J, Li C, Smith SM (Eds.) Hormone metabolism and signaling in plants. Academic Press. 2017;39-76.
3. Feng J, Shi Y, Yang S, Zuo J. 3 - Cytokinins, in: Li J, Li C, Smith SM. (Eds.) Hormone metabolism and signaling in plants. Academic Press. 2017;77-106.
4. Gao X, Zhang Y, He Z, Fu X. 4 - Gibberellins, in: Li J, Li C, Smith SM. (Eds.) Hormone metabolism and signaling in plants. Academic Press. 2017;107-160.
5. Li J, Wu Y, Xie Q, Gong Z. 5 - Abscisic acid, in: Li J, Li C, Smith SM. (Eds.) Hormone Metabolism and signaling in plants. Academic Press. 2017;161-202.
6. Hao D, Sun X, Ma B, Zhang JS, Guo H. 6 - Ethylene, in: Li J, Li C, Smith SM. (Eds.) Hormone metabolism and signaling in plants. Academic Press. 2017;203-241.
7. Wang H, Wei Z, Li J, Wang X. 9 - Brassinosteroids, in: Li J, Li C, Smith SM. (Eds.) Hormone metabolism and signaling in plants. Academic Press. 2017;291-326.
8. Jin JB, Cai B, Zhou JM. 8 - Salicylic acid, in: Li J, Li C, Smith SM. (Eds.) Hormone metabolism and signaling in plants. Academic Press. 2017;273-289.
9. Zhai Q, Yan C, Li L, Xie D, Li C. 7 - Jasmonates, in: Li J, Li C, Smith SM. (Eds.) Hormone metabolism and signaling in plants. Academic Press. 2017;243-272.
10. Wang B, Wang Y, Li J. 10 - Strigolactones, in: Li J, Li C, Smith SM. (Eds.) Hormone metabolism and signaling in plants. Academic Press. 2017;327-359.
11. Song XF, Ren SC, Liu CM. 11 - Peptide hormones, in: Li J, Li C, Smith SM. (Eds.) Hormone metabolism and signaling in plants. Academic Press. 2017;361-404.
12. Su Y, Xia S, Wang R, Xiao L. 13 - Phytohormonal quantification based on biological principles, in: Li J, Li C, Smith SM. (Eds.) Hormone metabolism and signaling in plants. Academic Press. 2017;431-470.
13. Chu J, Fang S, Xin P, Guo Z, Chen Y. 14 - Quantitative analysis of plant hormones based on LC-MS/MS. in: Li J, Li C, Smith SM. (Eds.) Hormone metabolism and signaling in plants. Academic Press. 2017;471-537.

14. Pan X, Wang X. Profiling of plant hormones by mass spectrometry. J. Chromatogr. B. 2009;877:2806-2813.
15. Li YH, Wei F, Dong XY, Peng JH, Liu SY, Chen H. Simultaneous analysis of multiple endogenous plant hormones in leaf tissue of oilseed rape by solid-phase extraction coupled with high-performance liquid chromatography–electrospray ionisation tandem mass spectrometry. Phytochem. Anal. 2011;22:442-449.
16. Han Z, Liu G, Rao Q, Bai B, Zhao Z, Liu H, Wu A. A liquid chromatography tandem mass spectrometry method for simultaneous determination of acid/alkaline phytohormones in grapes, J. Chromatogr. B. 2012;881-882:83-89.
17. Chu JF, Fang S, Xin PY, Guo ZP, Chen Y. Quantitative analysis of plant hormones based on LC-MS/MS, in: Li JY, Li CY, Smith SM. (Eds.) Hormone metabolism and signaling in plants. Academic Press. 2017;471-537.
18. Kenrick P, Crane PR. The origin and early evolution of plants on land. Nature. 1997;389:33-39.
19. Lu YD, Xu J. Phytohormones in microalgae: A new opportunity for microalgal biotechnology?. Trends Plant Sci. 2015;20:273-282.
20. Prieto REC, Cordoba CNM, Montenegro AMJ, González-Mariño GE. Production of indole-3-acetic acid in the culture medium of microalga *Scenedesmus obliquus* (UTEX 393). J. Braz. Chem. Soc. 2011;22:2355-2361.
21. Stirk WA, Balint P, Tarkowska D, Novak O, Maroti G, Ljung K, Tureckova V, Strnad M, Ordog V, van Staden J. Effect of light on growth and endogenous hormones in *Chlorella minutissim*a (Trebouxiophyceae). Plant Physiol. Biochem. 2014;79:66-76.
22. Stirk WA, Balint P, Tarkowska D, Novak O, Strnad M, Ordog V, van Staden J. Hormone profiles in microalgae: Gibberellins and brassinosteroids. Plant Physiol. Biochem. 2013;70:348-353.
23. Stirk WA, Novák O, Hradecká V, Pěnčík A, Rolčík J, Strnad M, Van Staden J. Endogenous cytokinins, auxins and abscisic acid in *Ulva fasciata* (Chlorophyta) and *Dictyota humifusa* (Phaeophyta): Towards understanding their biosynthesis and homoeostasis. Eur. J. Phycol. 2009;44:231-240.
24. Stirk WA, Ördög V, Novák O, Rolčík J, Strnad M, Bálint P, van Staden J. Auxin and cytokinin relationships in 24 microalgal strains. J. Phycol. 2013;49:459-467.
25. Stirk WA, Ördög V, Van Staden J, Jäger K. Cytokinin- and auxin-like activity in Cyanophyta and microalgae. J. Appl. Phycol. 2002;14:215-221.
26. Stirk WA, van Staden J, Novák O, Doležal K, Strnad M, Dobrev PI, Sipo G, Ördög V, Bálint P. Changes in endogenous cytokinin concentrations in *Chlorella* (Chlorophyceae) in relation to light and the cell cycle, J. Phycol. 2011;47:291-301.
27. Hirsch R, Hartung W, Gimmler H. Abscisic acid content of algae under stress, Botanica Acta. 1989;102:326-334.
28. Žižková E, Kubeš M, Dobrev PI, Přibyl P, Šimura J, Zahajská L, Záveská Drábková L, Novák O, Motyka V. Control of cytokinin and auxin homeostasis in cyanobacteria and algae, Ann. Bot. 2017;119:151-166.
29. Lu Y, Tarkowská D, Turečková V, Luo T, Xin Y, Li J, Wang Q, Jiao N, Strnad M, Xu J. Antagonistic roles of abscisic acid and cytokinin during response to nitrogen depletion in oleaginous microalga *Nannochloropsis oceanica* expand the evolutionary breadth of phytohormone function. Plant J. 2014;80:52-68.
30. Chokshi K, Pancha I, Ghosh A, Mishra S. Nitrogen starvation-induced cellular crosstalk of ROS-scavenging antioxidants and phytohormone enhanced the biofuel potential of green microalga *Acutodesmus dimorphus*, Biotechnol. Biofuels. 2017;10:60.
31. Mazhar S, Cohen JD, Hasnain S. Auxin producing non-heterocystous cyanobacteria and their impact on the growth and endogenous auxin homeostasis of wheat, J. Basic Microbiol. 2013;53:996-1003.
32. Hussain A, Krischke M, Roitsch T, Hasnain S. Rapid determination of cytokinins and auxin in cyanobacteria, Curr. Microbiol. 2010;61:361-369.
33. Sergeeva E, Liaimer A, Bergman B. Evidence for production of the phytohormone indole-3-acetic acid by cyanobacteria, Planta. 2002;215:229-238.

34. Tsavkelova EA, Klimova SY, Cherdyntseva TA, Netrusov AI. Hormones and hormone-like substances of microorganisms: A review, Appl. Biochem. Microbiol. 2006;42:229-235.
35. Maršálek B, Zahradníčková H, Hronková M. Extracellular abscisic acid produced by cyanobacteria under salt stress, J. Plant Physiol. 1992;139:506-508.
36. Boyer GL, Dougherty SS. Identification of abscisic acid in the seaweed *Ascophyllum nodosum.* Phytochem. 1988;27:1521-1522.
37. Tsavkelova EA, Klimova SY, Cherdyntseva TA, Netrusov AI. Microbial producers of plant growth stimulators and their practical use: A review. Appl. Biochem. Microbiol. 2006;42:117-126.
38. Gupta AB, Agarwal PR. Extraction, isolation, and bioassay of a gibberellin-like substance from phormidium foveolarum. Ann. Bot. 1973;37:737-741.
39. Le Bail A, Billoud B, Kowalczyk N, Kowalczyk M, Gicquel M, Le Panse S, Stewart S, Scornet D, Cock JM, Ljung K, Charrier B. Auxin metabolism and function in the multicellular brown alga *Ectocarpus siliculosus*, Plant Physiol. 2010;153:128-144.
40. Mazur H, Konop A, Synak R. Indole-3-acetic acid in the culture medium of two axenic green microalgae, J. Appl. Phycol. 2001;13:35-42.
41. Ashen JB, Cohen JD, Goff LJ. GC-SIM-MS detection and quantification of free indole-3-acetic acid in bacterial galls on the marine alga *Prionitis lanceolata* (Rhodophyta). J. Phycol. 1999;35:493-500.
42. Saradhi PP, Suzuki I, Katoh A, Sakamoto A, Sharmila P, Shi DJ, Murata N. Protection against the photo-induced inactivation of the photosystem II complex by abscisic acid, Plant, Cell Environ. 2000;23:711-718.
43. Yokoya NS, Stirk WA, van Staden J, Novák O, Turečková V, Pěnčík A, Strnad M. Endogenous cytokinins, auxins, and abscisic acid in red algae from Brazil, J. Phycol. 2010;46:1198-1205.
44. Watanabe T, Kondo N. Ethylene evolution in marine algae and a proteinaceous inhibitor of ethylene biosynthesis from red alga, Plant and Cell Physiology. 1976;17:1159-1166.
45. Kreslavsky VD, Kobzar EF, Muzafarov EN. Effect of red radiation, kinetin and linuron on growth and ethylene production in *Chlorella.* Biol. Plant. 1997;39:427-430.
46. Hartung W. The evolution of abscisic acid (ABA) and ABA function in lower plants, fungi and lichen, Funct. Plant Biol. 2010;37:806-812.
47. Zahradníčková H, Maršálek B, Polišenská M. High-performance thin-layer chromatographic and high-performance liquid chromatographic determination of abscisic acid produced by cyanobacteria. J. Chromatogr. 1991;555:239-245.
48. Featonby-Smith BC, Staden JV. Identification and seasonal variation of endogenous cytokinins in ecklonia maxima (Osbeck) Papenf. Bot. Mar. 1984;27:527-530.
49. Stirk WA, Novák O, Strnad M, van Staden J. Cytokinins in macroalgae, Plant Growth Regul. 2003;41:13-24.
50. Jennings R. Cytokinins as endogenous growth regulators in the algae *Ecklonia* (Phaeophyta) and Hypnea (Rhodophyta). Australian Journal of Biological Sciences. 1969;22:621-628.
51. Jennings, Gibberellins as endogenous growth regulators in green and brown algae. Planta. 1968;80:34-42.
52. Jennings RC, McCoMb AJ. Gibberellins in the Red Alga Hypnea musciformis (Wulf.) Lamour, Nature. 1967;215:872-873.
53. Ji X, Cheng J, Gong D, Zhao X, Qi Y, Su Y, Ma W. The effect of NaCl stress on photosynthetic efficiency and lipid production in freshwater microalga—*Scenedesmus obliquus* XJ002. Sci. Total Environ. 2018;633:593-599.
54. Church J, Hwang JH, Kim KT, McLean R, Oh YK, Nam B, Joo JC, Lee WH. Effect of salt type and concentration on the growth and lipid content of *Chlorella vulgaris* in synthetic saline wastewater for biofuel production, Bioresour. Technol. 2017;243:147-153.
55. Do TCV, Tran DT, Le TG, Nguyen QT. Characterization of endogenous auxins and gibberellins produced by *Chlorella sorokiniana* TH01 under phototrophic and mixtrophic cultivation modes toward applications in microalgal biorefinery and crop research. Journal of Chemistry. 2020;2020:4910621.

56. Du H, Liu H, Xiong L. Endogenous auxin and jasmonic acid levels are differentially modulated by abiotic stresses in rice, Front Plant Sci. 2013;4:397.
57. Du H, Wu N, Chang Y, Li X, Xiao J, Xiong L. Carotenoid deficiency impairs ABA and IAA biosynthesis and differentially affects drought and cold tolerance in rice, Plant Mol. Biol. 2013;83:475-488.
58. Colebrook EH, Thomas SG, Phillips AL, Hedden P. The role of gibberellin signalling in plant responses to abiotic stress, J. Exp. Biol. 2014;217:67-75.
59. Salama ES, Kabra AN, Ji MK, Kim JR, Min B, Jeon BH. Enhancement of microalgae growth and fatty acid content under the influence of phytohormones, Bioresour. Technol. 2014;172:97-103.
60. Liu J, Qiu W, Song Y. Stimulatory effect of auxins on the growth and lipid productivity of *Chlorella pyrenoidosa* and *Scenedesmus quadricauda.* Algal Res. 2016;18:273-280.
61. Sulochana SB, Arumugam M. Influence of abscisic acid on growth, biomass and lipid yield of *Scenedesmus quadricauda* under nitrogen starved condition, Bioresour. Technol. 2016;213:198-203.
62. Tietz A, Ruttkowski U, Kohler R, Kasprik W. Further investigations on the occurrence and the effects of abscisic acid in algae. Biochemie und Physiologie der Pflanzen. 1989;184:259-266.
63. Lee YK, Ding SY, Hoe CH, Low CS. Mixotrophic growth of *Chlorella sorokiniana* in outdoor enclosed photobioreactor, J. Appl. Phycol. 1996;8:163-169.
64. Kim S, Park JE, Cho YB, Hwang SJ. Growth rate, organic carbon and nutrient removal rates of *Chlorella sorokiniana* in autotrophic, heterotrophic and mixotrophic conditions. Bioresour. Technol. 2013;144:8-13.
65. Li T, Zheng Y, Yu L, Chen S. Mixotrophic cultivation of a *Chlorella sorokiniana* strain for enhanced biomass and lipid production. Biomass Bioenergy. 2014;66:204-213.
66. Jirásková D, Poulíčková A, Novak O, Sedláková K, Turečková V, Strnad M. High-throughput screening technology for monitoring phytohormone production in microalgae. J. Phycol. 2009;45:108-118.
67. Tran DT, Van Do TC, Nguyen QT, Le TG. Simultaneous removal of pollutants and high value biomaterials production by *Chlorella variabilis* TH03 from domestic wastewater. Clean Technol. Envir; 2020.
68. Mulders KJ, Lamers PP, Martens DE, Wijffels RH. Phototrophic pigment production with microalgae: biological constraints and opportunities. J. Phycol. 2014;50:229-242.
69. Perez-Garcia O, Escalante FME, de-Bashan LE, Bashan Y. Heterotrophic cultures of microalgae: Metabolism and potential products, Water Res. 2011;45:11-36.
70. Ende SSW, Noke A. Heterotrophic microalgae production on food waste and by-products, J. Appl. Phycol. 2019;31:1565-1571.
71. Nagarajan D, Lee DJ, Chang JS. Heterotrophic microalgal cultivation. in: Liao Q, Chang JS, Herrmann C, Xia A. Eds. Bioreactors for microbial biomass and energy conversion. Springer Singapore, Singapore. 2018;117-160.
72. Barros A, Pereira H, Campos J, Marques A, Varela J, Silva J. Heterotrophy as a tool to overcome the long and costly autotrophic scale-up process for large scale production of microalgae, Scientific Reports. 2019;9:13935.
73. Tran DT, Nguyen HY, Do TCV, Show PL, Le TG, Nguyen VT. Factors affecting pollutants removal and biomass production capability of *Chlorella variabilis* TH03 in domestic wastewater. Materials Science for Energy Technologies. 2020;3:545-558.
74. Narala RR, Garg S, Sharma KK, Thomas-Hall SR, Deme M, Li Y, Schenk PM. Comparison of microalgae cultivation in photobioreactor, open raceway pond, and a two-stage hybrid system. Frontiers in Energy Research. 2016;4.
75. Posten C. Design principles of photo-bioreactors for cultivation of microalgae. Eng. Life Sci. 2009;9:165-177.
76. Barceló-Villalobos M, Guzmán Sánchez JL, Martín Cara I, Sánchez Molina JA, Acién Fernández FG. Analysis of mass transfer capacity in raceway reactors. Algal Res. 2018;35:91-97.

77. Schädler T, Caballero Cerbon D, de Oliveira L, Garbe D, Brück T, Weuster-Botz D. Production of lipids with Microchloropsis salina in open thin-layer cascade photobioreactors, Bioresour. Technol. 2019;289:121682.
78. Wen X, Tao H, Peng X, Wang Z, Ding Y, Xu Y, Liang L, Du K, Zhang A, Liu C, Geng Y, Li Y. Sequential phototrophic–mixotrophic cultivation of oleaginous microalga *Graesiella* sp. WBG-1 in a 1000 m2 open raceway pond. Biotechnol. Biofuels. 2019;12:27.
79. Do TCV, Nguyen TNT, Tran DT, Le TG, Nguyen VT. Semi-continuous removal of nutrients and biomass production from domestic wastewater in raceway reactors using *Chlorella variabilis* TH03-bacteria consortia. Environmental Technology & Innovation. 2020;20:101172.
80. Doucha J, Lívanský K. Outdoor open thin-layer microalgal photobioreactor: Potential productivity. J. Appl. Phycol. 2009;21:111-117.
81. Commercial Production of Microalgae via Fermentation. Handbook of Microalgal Culture. 2013;134-145.
82. Shahid A, Malik S, Zhu H, Xu J, Nawaz MZ, Nawaz S, Asraful Alam M, Mehmood MA. Cultivating microalgae in wastewater for biomass production, pollutant removal, and atmospheric carbon mitigation; A review. Sci. Total Environ. 2020;704 :135303.
83. Kholssi R, Marks EAN, Miñón J, Montero O, Debdoubi A, Rad C, Biofertilizing effect of *Chlorella sorokiniana* suspensions on wheat growth. J. Plant Growth Regul. 2018;38:644-649.
84. Ding J, Mao LJ, Wang ST, Yuan BF, Feng YQ. Determination of endogenous brassinosteroids in plant tissues using solid-phase extraction with double layered cartridge followed by high-performance liquid chromatography–tandem mass spectrometry, Phytochem. Anal. 2013;24:386-394.
85. Chiwocha SDS, Abrams SR, Ambrose SJ, Cutler AJ, Loewen M, Ross ARS, Kermode AR. A method for profiling classes of plant hormones and their metabolites using liquid chromatography-electrospray ionization tandem mass spectrometry: An analysis of hormone regulation of thermodormancy of lettuce (*Lactuca sativa* L.) seeds, Plant J. 2003;35:405-417.
86. Li Y, Zhou CX, Yan XJ, Zhang JR, Xu JL. Simultaneous analysis of ten phytohormones in *Sargassum horneri* by high-performance liquid chromatography with electrospray ionization tandem mass spectrometry. J. Sep. Sci. 2016;39:1804-1813.
87. Mori IC, Ikeda Y, Matsuura T, Hirayama T, Mikami K. Phytohormones in red seaweeds: A technical review of methods for analysis and a consideration of genomic data, Bot. Mar. 2017;60:153-170.
88. Kojima M, Kamada-Nobusada T, Komatsu H, Takei K, Kuroha T, Mizutani M, Ashikari M, Ueguchi-Tanaka M, MatsuokaM, Suzuki K, Sakakibara H. Highly sensitive and high-throughput analysis of plant hormones using MS-probe modification and liquid chromatography-tandem mass spectrometry: an application for hormone profiling in *Oryza sativa.* Plant Cell Physiol. 2009;50:1201-1214.
89. Li YH, Wei F, Dong XY, Peng JH, Liu SY, Chen H. Simultaneous analysis of multiple endogenous plant hormones in leaf tissue of oilseed rape by solid-phase extraction coupled with high-performance liquid chromatography–electrospray ionisation tandem mass spectrometry, Phytochem. Anal. 2011;22:442-449.
90. Liu Z, Yuan BF, Feng YQ. Tandem phase extraction followed by online trapping–hydrophilic interaction chromatography–tandem mass spectrometry for sensitive detection of endogenous cytokinins in plant tissues, Phytochem. Anal. 2012;23:559-568.
91. Pan X, Welti R, Wang X. Simultaneous quantification of major phytohormones and related compounds in crude plant extracts by liquid chromatography-electrospray tandem mass spectrometry, Phytochem. 2008;69:1773-1781.
92. Blechschmidt S, Castel U, Gaskin P, Hedden P, Graebe JE, MacMillan J. GC/MS analysis of the plant hormones in seeds of *Cucurbita maxima.* Phytochem. 1984;23:553-558.
93. Tansupo P, Suwannasom P, Luthria DL, Chanthai S, Ruangviriyachai C. Optimised separation procedures for the simultaneous assay of three plant hormones in liquid biofertilisers. Phytochem. Anal. 2010;21:57-162.
94. Barone V, Baglieri A, Stevanato P, Broccanello C, Bertoldo G, Bertaggia M, Cagnin M, Pizzeghello D, Moliterni VMC, Mandolino G, Fornasier F, Squartini A, Nardi S, Concheri G. Root

morphological and molecular responses induced by microalgae extracts in sugar beet (*Beta vulgaris* L.). J. Appl. Phycol. 2018;30:1061-1071.

95. Ronga D, Biazzi E, Parati K, Carminati D, Carminati E, Tava A. Microalgal biostimulants and biofertilisers in crop productions, Agronomy. 2019;9:192.
96. Mehar NMUJG, Mudliar SN, Shekh AY. Recent advances in microalgal bioactives for food, feed, and healthcare products: commercial potential, market space, and sustainability. Compr. Rev. Food Sci. Food Saf. 2019;18:1882-1897.
97. Yan N, Fan C, Chen Y, Hu Z. The potential for microalgae as bioreactors to produce pharmaceuticals, Int. J. Mol. Sci. 2016;17:962.
98. Nicoletti M. Microalgae nutraceuticals. Foods. 2016;5:54.
99. Singh J, Gu S. Commercialization potential of microalgae for biofuels production, Renew. Sust. Energ. Rev. 2010;14:2596-2610.
100. Wei L, Wang Q, Xin Y, Lu Y, Xu J. Enhancing photosynthetic biomass productivity of industrial oleaginous microalgae by overexpression of RuBisCO activase, Algal Res. 2017;27:366-375.
101. Sero ET, Siziba N, Bunhu T, Shoko R, Jonathan E. Biophotonics for improving algal photobioreactor performance: A review, Int. J. Energy Res. 2020;1-22.
102. Karemore A, Ramalingam D, Yadav G, Subramanian G, Sen R. Photobioreactors for Improved algal biomass production: Analysis and design considerations, in: D. Das (Ed.) Algal Biorefinery: An Integrated Approach, Springer International Publishing, Cham. 2015;103-124.
103. Zhao P, Lin Z, Wang Y, Chai H, Li Y, He L, Zhou J. Facilitating effects of plant hormones on biomass production and nutrients removal by *Tetraselmis cordiformis* for advanced sewage treatment and its mechanism. Sci. Total Environ. 2019;693:133650.
104. Liu J, Qiu W, Song Y, Peng H, Zhao Y. The growth and lipid productivity of *Chlorella pyrenoidosa* enhanced by plant hormones under ammonium stress. Environ. Prog. Sus. Energ. 2017;36:1187-1193.
105. Garcia-Gonzalez J, Sommerfeld M. Biofertilizer and biostimulant properties of the microalga *Acutodesmus dimorphus.* J. Appl. Phycol. 28 (2016) 1051-1061.
106. By Onder Uysal, Ozge Uysal F, Ekinci K. Evaluation of microalgae as microbial fertilizer, Eur. J. Sustain. Dev. 2015;4:77-82.
107. Faheed F, Fattah Z. Effect of *Chlorella vulgaris* as bio-fertilizer on growth parameters and metabolic aspects of Lettuce plant, J Agric Soc Sci. 2008;4:165-169.
108. Choleva B, Bileva T, Tzvetkov Y, Barakov P. Preliminary study of the green algae chlorella (*Chlorella vulgaris*) for control on the root-knot nematode (*Meloidogyne arenaria*) in tomato plants and ectoparasite Xiphinema indexin grape seedlings, Commun. Agric. Appl. Biol. Sci. 2005;70:915-926.
109. Oancea F, Velea S, Fatu V, Mincea C, Ilie L. Micro-algae based plant biostimulant and its effect on water stressed tomato plants. Rom. J. Plant Prot. 2013;6:104-117.
110. Areeg Abd Elhafiz SSG, Afnan Abd Elhafiz, Noor Hamdany, Maryam Osman, Lakshmi TVR. *Chlorella vulgaris* and *Chlorella pyrenoidosa* live cells appear to be promising sustainable biofertilizer to grow rice, lettuce, cucumber and eggplant in the UAE soils. Recent Research in Science and Technology. 2015;7:14-21.
111. Kholssi R, Marks EAN, Miñón J, Montero O, Debdoubi A, Rad C. Biofertilizing effect of *Chlorella sorokiniana* suspensions on wheat growth. J. Plant Growth Regul. 2019;38:644-649.
112. Kim MJ, Shim CK, Kim YK, Ko BG, Park JH, Hwang SG, Kim BH. Effect of biostimulator *Chlorella fusca* on improving growth and qualities of Chinese Chives and Spinach in organicfarm, Plant Pathol. J. 2018;34:567-574.
113. Puglisi I, La Bella E, Rovetto EI, Lo Piero AR, Baglieri A. Biostimulant effect and biochemical response in lettuce seedlings treated with a *Scenedesmus quadricauda* extract. Plants (Basel) 2020;9.
114. Mutale-Joan C, Redouane B, Najib E, Yassine K, Lyamlouli K, Laila S, Zeroual Y, Hicham EA. Screening of microalgae liquid extracts for their bio stimulant properties on plant growth, nutrient uptake and metabolite profile of *Solanum lycopersicum* L. Sci Rep. 2020;10:2820.

115. Liu X, Zhang H, Zhao Y, Feng Z, Li Q, Yang HQ, Luan S, Li J, He ZH. Auxin controls seed dormancy through stimulation of abscisic acid signaling by inducing ARF-mediated ABI3 activation in *Arabidopsis*, Proc Natl Acad Sci USA. 2013;110:15485-15490.
116. Shuai H, Meng Y, Luo X, Chen F, Zhou W, Dai Y, Qi Y, Du J, Yang F, Liu J, Yang W, Shu K. Exogenous auxin represses soybean seed germination through decreasing the gibberellin/abscisic acid (GA/ABA) ratio, Scientific Reports. 2017;7:12620.
117. Guangwu Z, Xuwen J. Roles of gibberellin and auxin in promoting seed germination and seedling vigor in *Pinus massoniana.* For. Sci. 2013;60:367-373.
118. Koutra E, Tsafrakidou P, Sakarika M, Kornaros M. Chapter 11 - Microalgal Biorefinery, in: A. Yousuf (Ed.) Microalgae cultivation for biofuels production. Academic Press. 2020;163-185.
119. Callejo-López JA, Ramírez M, Bolívar J, Cantero D. Main variablesaffecting a chemical-enzymatic method to obtain protein and amino acids from resistant microalgae, Journal of Chemistry. 2019;2019:1-10.
120. Hayes M, Skomedal H, Skjånes K, Mazur-Marzec H, Toruńska-Sitarz A, Catala M, Isleten Hosoglu M, García-Vaquero M. 15 - Microalgal proteins for feed, food and health, in: C. Gonzalez-Fernandez, R. Muñoz (Eds.) Microalgae-Based Biofuels and Bioproducts, Woodhead Publishing. 2017;347-368.
121. Khoo KS, Chew KW, Yew GY, Leong WH, Chai YH, Show PL, Chen WH. Recent advances in downstream processing of microalgae lipid recovery for biofuel production, Bioresour. Technol. 2020;122996.
122. de Morais MG, Vaz Bda S, de Morais EG, Costa JA. Biologically active metabolites synthesized by microalgae, Biomed Res Int; 2015;2015:835761.
123. Barone V, Puglisi I, Fragalà F, Stevanato P, Baglieri A. Effect of living cells of microalgae or their extracts on soil enzyme activities, Arch. Agron. Soil Sci. 2019;65:712-726.
124. Tran DT, Lee HR, Jung S, Park MS, Yang JW. Lipid-extracted algal biomass based biocomposites fabrication with poly(vinyl alcohol). Algal Res. 2018;31:525-533.

Biography of author(s)



Cam Van T. Do
Graduate University of Science and Technology, Vietnam Academy of Science and Technology, 18 Hoang Quoc Viet, Cau Giay, Ha Noi, Vietnam and Center for Research and Technology Transfer, Vietnam Academy of Science and Technology, 18 Hoang Quoc Viet, Cau Giay, Ha Noi, Vietnam and Department of Chemical Technology, Hanoi University of Industry, No. 298 Cau Dien, Bac Tu Liem, Ha Noi, Vietnam.

Research and Academic Experience: PhD at Bonn University, Germany (2012).
Postdoc at Graduate University of Science and Technology, Vietnam (2017-2020)
She is a lecturer at Faculty of Chemical Technology, Hanoi University of Industry, Vietnam.
She is an expert in environmental and agricultural sciences. She has published more than 10 peer-reviewed articles (ISI, SCI), 01 book and 02 book chapters. She is also a principal investigator of more than 2 research funding and a participator of more than other 5 research projects.

Research Area: Microalgae biotechnology, Microalgae biomass, microalgae cultivation, wastewater treatment using microalgae, CO2 sequestration using microalgae, microalgae-based biorefinery.

Number of Published papers: 10 papers



Thuan Dang Tran
Institute of Chemistry, Vietnam Academy of Science and Technology, 18 Hoang Quoc Viet, Cau Giay, Ha Noi, Vietnam.

Research and Academic Experience: Ph.D at Department of Chemical Engineering, National Cheng Kung University, Taiwan (2013).
Postdoc at Korea Advanced Instituted of Science and Technology, Korea (2014-2016)
He is vice director of Department of Green Chemistry, Institute of Chemistry, Vietnam Academy of Science and Technology
He has over 10 years' experience research in microalgae. He has published more than 24 peer-reviewed articles (SCI), 02 book chapters, 02 patents. He is also a principal investigator of more than 4 research funding.

Research Area: Microalgae biotechnology, Microalgae biomass, microalgae cultivation, wastewater treatment using microalgae, CO_2 sequestration using microalgae, microalgae-based biorefinery, biofuels, lignocellulose processing.

Number of Published papers: 24 papers



Quang Trung Nguyen
Graduate University of Science and Technology, Vietnam Academy of Science and Technology, 18 Hoang Quoc Viet, Cau Giay, Ha Noi, Vietnam and Center for Research and Technology Transfer, Vietnam Academy of Science and Technology, 18 Hoang Quoc Viet, Cau Giay, Ha Noi, Vietnam.

Research and Academic Experience: Ph.D at University of Poitiers, France, in Environmental Technology (2007).
He is Director of Center for Research and Technology Transfer, Vietnam Academy of Science and Technology, Vietnam.
He has over 12 years' experience research in environmental sciences and technology. He has published more than 26 peer-reviewed articles (SCI), 05 books, 03 patents. He is also a principal investigator of more than 11 research funding.

Research Area: Air pollution monitoring and research, food safety & sciences research, dioxins and other environmental toxics, analysis of environmental and food toxics.

Number of Published papers: 20 papers

© Copyright (2021): Author(s). The licensee is the publisher (Book Publisher International).

DISCLAIMER
This chapter is an extended version of the article published by the same author(s) in the following journal.
Journal of Chemistry, 2020: 1-11, 2020.

Chapter 4
Print ISBN: 978-93-90516-93-3, eBook ISBN: 978-93-90516-94-0

Liver Enzymes and HIV-infected Adults on HARRT: Evidence from a Randomised Cross-sectional Study in UPTH

Chinwe F. Anyanwu[1] and Eric O. Aigbogun Jr.[2*]

DOI: 10.9734/bpi/rrab/v5/2065E

ABSTRACT

This study evaluated the liver enzyme levels of HIV-infected adult patients on highly active antiretroviral treatment (HAART) for not less than 1 year. The study was designed as a randomised cross-sectional study that evaluated the differences in the aspartate aminotransferase [AST], alanine transaminase [ALT] and alkaline phosphatase [ALP] of 129 (46 males and 83 females) HIV-infected adult patients. Before the study, ethical clearance (Ref: UPH/CEREMAD/REC/19) was obtained from the University of Port Harcourt Ethics Committee. Venous blood samples were obtained and the liver enzyme levels were analysed using Clinical Chemistry Analyser (VS10) manufactured by Vitro Scient. Values were further categorised into; normal or abnormal. SPSS version 21 (IBM® Armonk, USA) and Graph Pad Prism (Version 8.0.2) was used to analyse the data. T-test compared the sex differences in mean, while Chi-square analysis tested the sex differences in the categorised data. From the result, the mean AST (27.65±17.93 IU/L) and ALP (135.13±10.87 IU/L) values for males were higher than females AST (27.25±18.93 IU/L) and ALP (132.65±9.96 IU/L) values, while ALT was higher in female (34.66±22.29 IU/L) than males (33.75±18.14 IU/L); however, the differences were not significant ($p>0.05$). Generally, abnormal AST, ALT and ALP levels were 31%, 34% and 82% respectively; with no sex-associated differences ($p>0.05$). 45.7% of the patients (males: 25 [54.3%] and females: 34 [41.0%]) had cholestatic abnormality, while 8.5% (5; 10.9% males and 6; 7.2% females) had hepatocellular abnormality, 18.6% (6; 13.0% of males and 18; 21.7% of females) and mixed abnormality (AST/ALT/ALP). The correlation between CD4 count and liver enzymes were not significant ($P>0.05$). In conclusion, cholestatic abnormalities were observed in more than 80% of the patients as opposed to hepatocellular abnormalities, which were less than 35%. More females were associated with mixed abnormality when compared to males with independent (ALT/AST) abnormality. This study, therefore, suggests the need for a randomised case-control study to highlight the extent of deviation from normal values.

Keywords: Enzyme levels; liver; HAART; HIV-infected adults; sex; CD4 count.

1. INTRODUCTION

1.1 The HIV/AIDS Pandemic

putting an end to the Human immunodeficiency virus and acquired immunodeficiency syndrome (HIV/AIDS) is still a global challenge, especially in under-developed and developing countries that struggle with medical facilities and intervention management system [1]. The declaration of HIV/AIDS as “a global emergency” was handed down by the United Nations General Assembly in June 2001. This resulted in the meeting of all Member States with the sole aim of preventing the disease and caring for the infected [1–3]. They suggested that if all measures were put in place, by 2005, there will be a 25% reduction in infection rates among 15–24 years old; even in the worst-affected countries

[1]*Department of Pharmacology, Faculty of Basic Clinical Sciences, University of Port Harcourt, Nigeria.*
[2]*Department of Anatomy, Faculty of Medical Sciences, Kampala International University, Western Campus, Uganda.*
**Corresponding author: E-mail: eric.aigbogun@kiu.ac.ug, eric_aigbogun@uniport.edu.ng;*

(and globally by 2010) and a 20% reduction in the number of infants infected with HIV by 2005 (and by 50% by 2010) [4]. Despite the declaration and measures, new infections are recorded yearly [3,5] and based on the current projections, the Global HIV/AIDS deaths are expected to rise from 2.8million in 2002 to 6.5 million in 2030 under the baseline scenario; based on the assumption that antiretroviral (ARV) drugs coverage reaches 80% by 2012. On the other hand, with optimistic projections, with the assumption that preventive activities increases, HIV/AIDS deaths are projected to drop to 3.7 million in 2030 [6]. The Sub-Saharan African Region has roughly 25 million people infected, [7,8] making it the highest region with the disease; of which Nigeria is a major contributor because of her population (Fig. 1) [9].

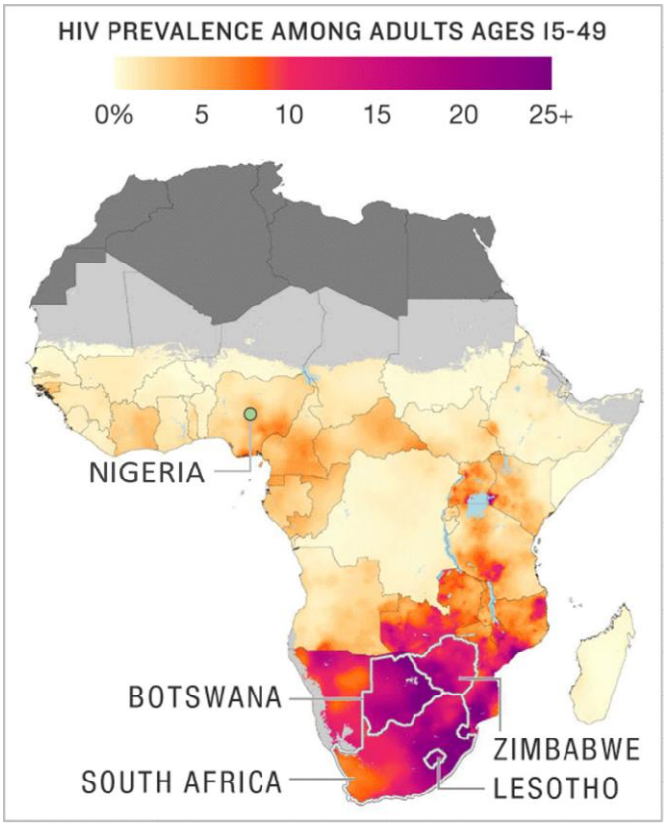


Fig. 1. Mapping of HIV prevalence in Sub-Saharan (*modified to indicate Nigeria*)[7]

1.2 Highly Active Antiretroviral Therapy (HAART)

The term HAART was introduced in the 1990s to describe the effectiveness of combination drug therapies used to treat HIV in infected patients [10], and ever since, it has brought about dramatic improvements in the survival of patients [11–14]. It is known that the therapies were not designed as a cure, rather, to control the viral load to as low as reasonably achievable; so that infected persons can have a shot at a longer, healthier life, as well as reduce the risk of transmitting HIV to others [11–13,15]. However, antiretroviral drugs have been associated with systemic toxicity (both short and long-term) [14].

1.3 Liver Enzymes (ALT, AST & ALP)

Alanine transaminase (ALT) is an enzyme found in the liver that helps convert proteins into energy for the liver cells. When the liver is damaged, ALT is released into the bloodstream and levels increase. Aspartate transaminase (AST) is an enzyme that helps metabolize amino acids, it is also normally

present in blood at low levels, and an increase may indicate liver damage, disease or muscle damage. Alkaline phosphatase (ALP) is an enzyme found in the liver and bone and is important for breaking down proteins. Higher-than-normal levels of ALP may indicate liver damage or diseases, such as a blocked bile duct, or certain bone diseases [16].

According to the AIDS Clinical Trials Group scale of liver toxicity, [17] hepatotoxicity in a patient is observed when the transaminases (ALT and/or AST) rise above the upper limits of the normal range. Severe hepatic injuries are classified as grade 3 or 4 changes in AST and/or ALT levels; that is ALT, AST levels are 3-5 and greater than 5 times the upper normal limit, respectively during treatment with ARVs. Also, severe hyperbilirubinaemia is classified as grade 3 or 4 and is treated independently of AST and ALT level [18].

1.4 HIV/AIDS, HAART, Hepatotoxicity and Liver Enzymes

ART associated liver disease is now the most common non-AIDS related cause of death among HIV-infected patients, accounting for 14-18% of all deaths in this population and almost half of the deaths among hospitalized HIV-infected patients [19,20]. Studies by Ngala et al. [21], Wambani et al. [22] and Neukam et al. [23] suggest that prolonged use of any class of ART is implicated in hepatotoxicity that is often seen in patients undergoing drug treatment; however, the extent to which each of these drugs induces or contributes to hepatotoxicity varies considerably [21].

The term Highly Active Antiretroviral Therapy (HAART) refers to the customized combination of three or more antiretroviral agents [24] which generally consists of a combination of Nucleoside Analog Reverse Transcriptase Inhibitors (NRTI) plus a Protease Inhibitor (PI) and Non-Nucleoside Reverse Transcriptase Inhibitors (NNRTI) [12,25]. The increased availability of the different combinations has been identified to have the potential to increase the hepatotoxicity susceptibility [26].

The hepatic system which is responsible for drug detoxification is greatly affected by HAART, [14] and it is very difficult to vary the extent of damage the individual drugs induce or contribute to hepatotoxicity; as some studies have shown that PI's (full-doses; ritonavir) were found to be intensely hepatotoxic [27–29]. While other findings suggest milder to no effects in some HARRT [22,30-32]. Indinavir (IDV) and saquinavir (SQV) has also been reported to be associated with several cases of liver damage [33,34]. Antiretroviral drug-related liver injury (ARLI) is common among HIV-patients and often results in the discontinuation of treatment, [35,36] and it is defined by elevations in serum hepatic enzymes; [37] with alanine aminotransferase (ALT) characteristically greater than aspartate aminotransferase (AST) [36]. The method of classifying the severity of hepatotoxicity in clinical studies varies. Some studies consider ALT parameters as minimal as two times the upper limits of normal [38] while others use absolute threshold (for example, >100 IU/ml), regardless of baseline liver function tests [39]. Nevertheless, the severity of ARLI may range from – no symptom to liver decompensation and the consequence could range from spontaneous resolution to liver failure and death [40,41].

There are shreds of evidence suggesting that when patients are placed on HAART, they will in the nearest future present with abnormal hepatic enzyme levels, [42,43] which may even lead to liver failure [40,44] However, co-infection with hepatitis B or C virus [14,39,41,45,46] and tuberculosis treatment [47,48] have also been implicated in mild to moderate hepatotoxicity, with significant elevation in ALT and AST levels [36]. In Tanzania, efavirenz and rifampicin-based hepatotoxicity occurred in HIV patients with or without tuberculosis (TB) co-infection, but the observed toxic effects were mild, hence, therapeutic modifications was not recommended [44]. Whereas, in a recent case-study by Segamwenge and Bernard [49] four patients were reported to have been diagnosed with acute liver failure within one month of switching to an efavirenz-based antiretroviral therapy. Studies found that up to 22% of HIV patients will have elevated enzymes levels upon commencement of ART [42,50–52], and the elevations could vary with associated coinfections [46,50,52,53].

1.5 HIV/AIDS, HAART, Liver Enzymes and CD4 Count

There is a paucity of data regarding the relationship between CD4+ count and liver enzymes. In a recent study [54], negative but no significant correlations were observed for ALT and AST in HIV monoinfected and co-infected groups.

It is suggested that sex-specific thresholds be applied when investigating liver enzyme levels because women have slightly lower normal ALT levels than men [55]. This was evident in a study conducted in the U.S. that identified an ALT upper limit of 29 IU/L for men and 22 IU/L for women [56]. Additionally, in the study by Wu et al. [57] suggesting that liver enzymes in the aetiology of diseases, abnormal elevations may differ by sex, it is therefore imperative that while investigating the liver enzyme levels among HIV-infected patients on HAART, sex-associated differences are evaluated.

2. MATERIALS AND METHODS

2.1 Research Design

This study was designed as a "cross-sectional" part of a 2016 randomized comparative study (CEREMAD) by Anyanwu et al. [58]. The study population involved HIV-positive patients, who were on HAART for not less than 1 year and whose CD4 count was not below the critical value of 200 cells/mm^3. Personal information such as age, sex, lifestyle (alcohol and cigarette consumption), pregnancy status for the females, duration of HAART and HIV co-infections (such as pulmonary tuberculosis, hepatitis B & C) history were obtained from their hospital folders and pre-tested questionnaires.

2.2 Study Population

The clinic had over twelve thousand (12,000) registered HIV patients. Data for 900 HIV-infected patients undergoing HAART at University of Port Harcourt Teaching Hospital as of 2015 were collected. However, 392 patients were physically present for recruitment.

2.3 Sampling Technique

This study utilized a randomised purposive sampling method.

2.4 Eligibility and Enrolment

1. Inclusion criteria - patients: (i) diagnosed with HIV infection and undergoing HAART; (ii) aged >20 years; (iii) continuing HAART (≥12 months), with baseline records indicating that their baseline serum ALT, AST, and ALP levels were within normal ranges (ALT=7-55U/L; AST=8-48U/L; ALP=40-129 U/L).
2. Exclusion criteria - patients (i) with acute HIV infection; with (ii) co-infections or severe, life-threatening complications; (iii) who were pregnant; (iv) with autoimmune diseases; (v) with incomplete data. (vi) older than 55 years of age [58–60].

2.5 Methods of Data (sample) Collection/Instrumentation

Venous blood samples were collected and serum levels of Aspartate aminotransferase [AST], Alanine transaminase [ALT] and Alkaline phosphatase [ALP] were determined using Clinical Chemistry Analyser (VS10) manufactured by Vitro Scient. The machine utilises the operational principle guided by Beer-lambert's law (that is; the linear relationship between absorbance and concentration of an absorbing species) [61]. Normal range values were established in units per litre as follows; ALT (7 to 55 U/L), AST (8 to 48 U/L) and ALP (40 to 129 U/L) [16]. CD4 T-cell count was estimated using FACSCalibur™ flow cytometer (Becton Dickinson, California, USA), [62] under standard protocol as described by the CDC [63].

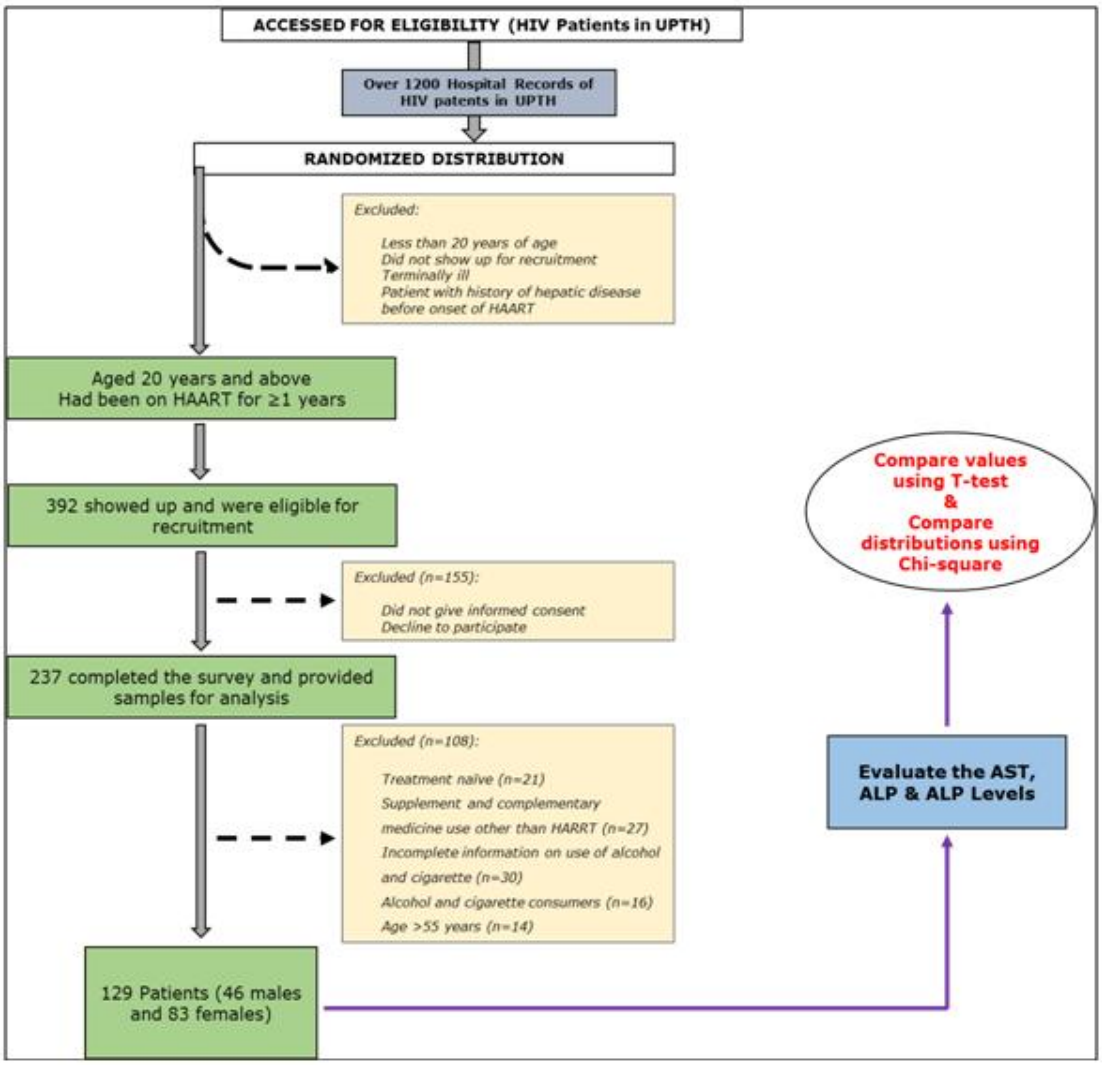


Fig. 2. Flow diagram for the research lifecycle

2.6 Data Analysis

Statistical Package for the Social Sciences (SPSS IBM® version 23), STATGRAPHICS centurion CVI version 16.1.11 (StatPoint Tech., Inc.), and GraphPad Prism (Version 8.0.2) were used in analysing the data. Descriptive statistics were performed for continuous data and represented as mean (S.D) while frequencies (%) were used to express categorical data. Z-test was used to determine the mean difference while Fisher's Chi-square analysis was used to determine trends and associations. The confidence level was set at 95% and a P-value less than 0.05 was considered significant.

3. RESULTS

The mean values of the hepatic enzyme markers presented in Table 1 showed that the mean(S.D) AST (27.65±17.93 U/L) and ALP (135.13±10.87 U/L) values for males were higher than the female values (AST = 27.25±18.93 U/L and ALP (132.65±9.96 U/L), whereas females (34.66±22.29 U/L) had higher ALT values than males (33.75±18.14 U/L). The mean CD4+ cell count was slightly lower in males 425.96±68.30 cells/mL than in females 442.31± 51.44 cells/mL. However, the t-test of mean differences was not significant (P>0.05) (Fig. 3).

Table 1. The mean, standard deviation of the liver enzymes of HIV-infected patients on HAART

Variables	Mean±S.D [95% C,I]	
	Male (n=46)	Female (n=83)
AST (U/L)	27.65±17.93 [22.323, 32.974]	27.25±18.93 [23.122, 31.388]
ALT (U/L)	33.75±18.14 [28.368, 39.14]	34.66±22.29 [29.55, 38.636]
ALP (U/L)	135.13±10.87 [131.9, 138.357]	132.65±9.96 [130.473, 134.821]
CD4 count cells/mL	425.96±68.30 [357.653, 494.26]	442.31± 51.44 [390.878, 493.748]

Note: Aspartate Aminotransferase [AST], Alanine Transaminase [ALT], Alkaline Phosphatase [ALP], Confidence Interval [C.I], Standard deviation [S.D]

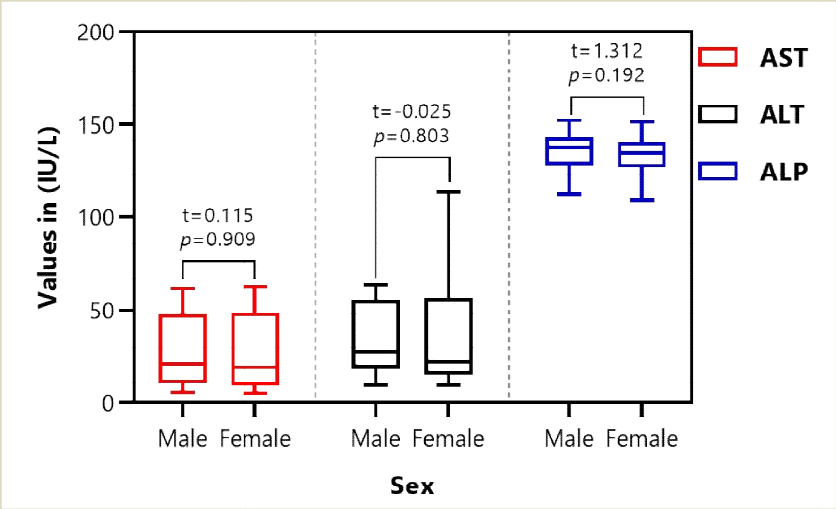


Fig. 3. Comparison of the mean AST, ALT, and ALP levels in males and female

The values obtained were categorized; within [normal] and outside normal limit [abnormal] and the Chi-square analysis of sex association were represented in Table 2. The result for AST and ALT showed that males patients (AST = 34; 73.9% and ALT = 31; 67.4%) were observed to have more normal values than females (AST = 55; 66.3% and ALT = 53; 63.9%). But slightly lower normal ALP values were observed for males (8; 17.4%) when compared to females (15; 18.10%). The differences in AST (χ^2=0.809; *p*=0.368), ALT (χ^2=0.163; *p*=0.686), and ALP (χ^2=0.009; *p*=0.923) were not statistically significant. For abnormal AST and ALT the odds were slightly lower in females; OR_{AST} [95% C.I]= 0.763 [0.416 1.399] and OR_{ALT} [95% C.I] = 0.902 [0.545 - 1.494], while there was no difference in risk for ALP (OR=1.058 [0.847 1.321]).

Table 2. Liver enzyme characteristics and test of sex-associated distributional test differences

		Normal (%)	Abnormal (%)	Chi-sq. (χ^2)	P-value	OR abnormal [95% C.I]
AST	Male	34 (73.9)	12 (26.1)	0.809	0.368	0.763 [0.416 1.399]
	Female	55 (66.3)	28 (33.7)			
	Total	89 (69.0)	40 (31.0)			
ALT	Male	31 (67.4)	15 (32.6)	0.163	0.686	0.902 [0.545 - 1.494]
	Female	53 (63.9)	30 (36.1)			
	Total	84 (65.1)	45 (34.9)			
ALP	Male	8 (17.4)	38 (82.60)	0.009	0.923	1.058 [0.847 1.321]
	Female	15 (18.10)	68 (81.90			
	Total	23 (17.8)	106 (82.2)			

Note: Aspartate Aminotransferase [AST], Alanine Transaminase [ALT], Alkaline Phosphatase [ALP]
Normal values: ALT (7 to 55 U/L), AST (8 to 48 U/L), and ALP (40 to 129 U/L)

Among the patients, 17.8% (6; 13.0% males and 17; 20.5% female) were normal, while no patient (male or female) had abnormal AST levels. 3; 2.3% of the patents had abnormal ALT levels. 59; 45.7% of the patients (males: 25 [54.3%] and females: 34 [41.0%]) had cholestatic abnormality (higher than normal ALP levels). For hepatocellular abnormality, 8.5% (5; 10.9% males and 6; 7.2% females) were observed for this group, while for mixed abnormality (AST/ALT/ALP), 18.6% (6; 13.0% of males and 18; 21.7% of females) fell into this class (Table 3).

There was a negative correlation between CD4 cell count and AST (r=-0.087, Rsq.=0.76%) and ALT (r=-0.10, Rsq.=0.99%) levels, but no correlation with ALP (r=0.0048, Rsq.=0.002%). However, the correlations were not significant (CD4 vs; AST [P=0.325], ALT [P=0.261], ALP [P=0.957]) (Fig. 4).

Table 3. Liver enzyme characteristics and associated coexistence of abnormalities

Hepatological condition	Sex		Total (%)
	Male (%)	Female (%)	
Normal	6 (13.0)	17 (20.5)	23 (17.8)
AST	-	-	-
ALT	1 (2.2)	2 (2.4)	3 (2.3)
ALP	25 (54.3)	34 (41.0)	59 (45.7)
AST/ALT	5 (10.9)	6 (7.2)	11 (8.5)
AST/ALP	-	2 (2.4)	2 (1.6)
ALT/ALP	3 (6.5)	4 (4.8)	7 (5.4)
AST/ALT/ALP	6 (13.0)	18 (21.7)	24 (18.6)

Note: Aspartate Aminotransferase [AST], Alanine Transaminase [ALT], Alkaline Phosphatase [ALP]

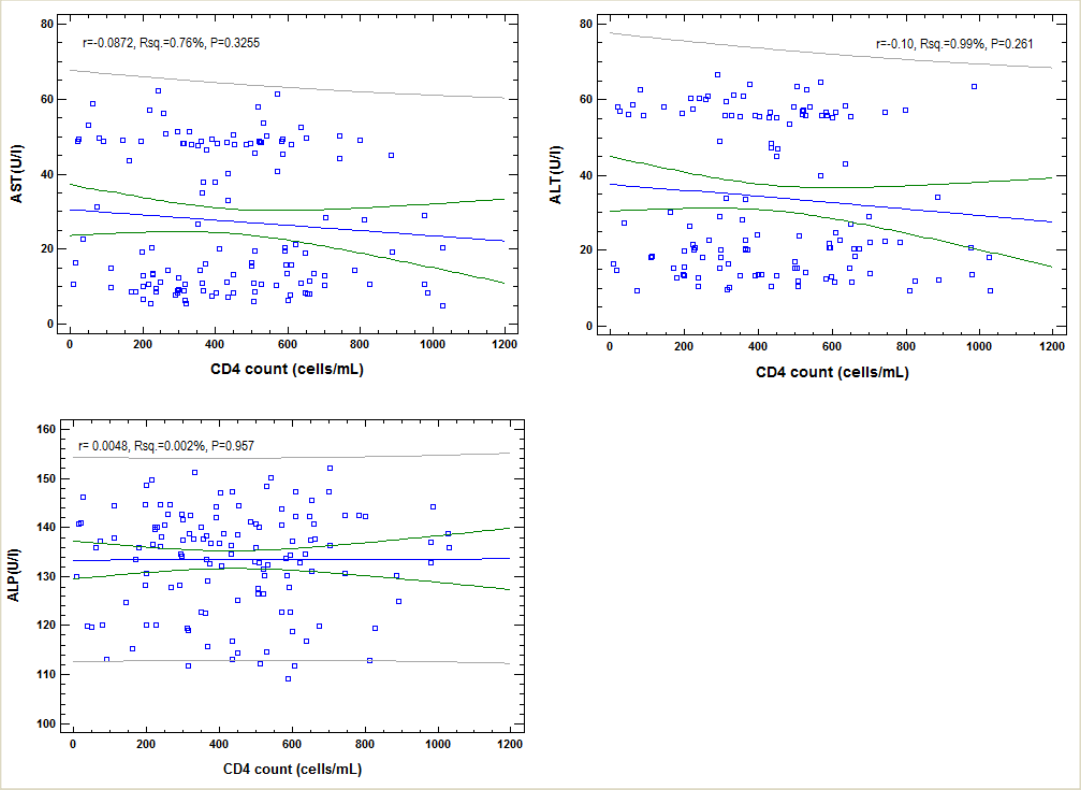


Fig. 4. Correlation of CD4 count with AST, ALT, and ALP levels among HIV-infected patients on HAART

4. DISCUSSION

Since the alarming rise in HAART-induced hepatotoxicity, prevention and management of ART-related toxicity has emerged as a major issue for HIV/AIDS treatment and care. In this study, the mean AST and ALT of male and female HIV patients were within the normal range, while the ALP values were way above the upper limit of 129 U/L for normal values [6]; however, more females had ALT and AST values that were above the normal upper limit reported by Ruhl and Everhart, 2012 [16]. The finding in this study agrees with previous observations; that several HIV patients will have elevated enzymes levels upon commencement of ART [42,50–52].

The findings in this study do not agree with the reports of more independent association with a mild increase in ALT and AST values; [52] as independent ALT and AST abnormality were less common when compared to both abnormalities (ALT/AST). On the other hand, ALP as an independent abnormality was the most observed; accounting for more than 40%. Predominant abnormal ALP levels in HIV patients without hepatitis co-infection has been reported [52,53]. In the study by Maida, [64,24] among 17 patients with increased liver enzymes, ALP was elevated in 16. Sterling et al. [53] also found that evaluated ALP was more common than AST or ALT. It is also important to note that mixed (hepatocellular + cholestatic abnormality) was commoner among females, while independently, the hepatocellular and cholestatic abnormality was more observed in males.

Several possible mechanisms through which HIV medications are capable of causing liver enzyme elevations, these include direct toxicity, hypersensitivity reactions, mitochondrial toxicity, and metabolic abnormalities have been observed from adverse drug reaction to HARRT. Direct liver toxicity arises when the drug is metabolized in the liver through the cytochrome pathways may cause liver toxicity when there are polymorphisms in the enzymes [65,30]. Particularly NNRTIs and PIs can cause and are affected by alterations of the cytochrome P450 enzymes in the liver [66]. Hypersensitivity is idiosyncratic and often associated with specific components of the drug (such as sulphas) and not the dose [67]. For example, Nevirapine (NVP) has been associated with about 4% of symptomatic adverse drug reactions in the liver [30]. Mitochondria toxicity arises when some ARVs inhibit mitochondrial DNA synthesis in vitro [68]. Evidence suggests that additive or synergistic long-term mitochondrial toxicity is associated with some NRTI combinations.[68,69] It is also noted that most HAART contains PIs which has been associated with the development of insulin resistance (IR) and dyslipidemia, both risk factors for steatosis [64,70–72]. Additionally, BMI > 30 and Diabetes Mellitus (DM) have been associated with increased liver enzymes in HIV patients on HARRT [52,23].

The complexity of medications used in HAART makes it difficult to understand the independent effects of each drug in the development of drug-induced liver injury [28,37]. This makes the prevention and management of antiretroviral therapy-related toxicity is a major issue for HIV/AIDS treatment and care [65,73]. The pathogenesis of drug-induced liver disease normally involves the participation of the parent drug or its metabolite that either affects the cell biochemistry directly or indirectly by eliciting an immune response [74]. As HIV-infected patients live longer, they develop long-term manifestations of chronic HIV infection and treatment complications [74]. The severity of ART may range from the absence of symptoms to liver decomposition and the outcome ranges from spontaneous resolution to liver failure and death [75].

In this study, the relationship between liver enzymes (AST, ALT and ALP) and CD4 count was investigated and it was found that AST and ALT were negatively but not significantly correlated with CD4 count as reported by Hooja et al. [54] while ALP had a very week (near zero) correlation with CD4.

The findings in this study do not categorically exclude sex influence in hepatic enzyme abnormalities in HIV-infected patients (without hepatitis co-infection), because the mechanism of action of HIV drugs in inducing hepatotoxicity may be significantly influenced by hormones (which vary with sex), and other clinical conditions such as BMI, diet type and DM, which are not directly associated with HIV infection.

5. CONCLUSION

Generally, among HIV-infected patients on HAART in UPTH, cholestatic abnormalities were observed in more than 80% of the patients as opposed to hepatocellular abnormalities, which were less than 35%. More females were associated with mixed abnormality when compared to males with independent (ALT/AST) abnormality. Any observed sex-associated difference could be associated with a series of unidentified mechanisms of action of HAART. Additionally, liver enzymes are not significantly associated with CD4+ parameters. This study, therefore, suggests the need for a randomised case-control study to highlight the extent of deviation from normal values.

Ultimately, there is a need for hospitals to design and implement timely intervention and management strategies; to reduce the rate of drug-induced liver damage in HIV-infected patients. Therefore, policymakers must develop better and newer management strategies; to reduce the burden associated with drug toxicity in HIV-infected patients.

CONSENT AND ETHICAL APPROVAL

Ethical approval with reference number UPH/CEREMAD/REC/18 was obtained from the Research Ethics Committee of the University of Port before the commencement of the research. The study was conducted in line with the Declaration of Helsinki, [76] and all other relevant statutory regulations were observed. Informed consent was obtained from all participating patients.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. [WHO. Global Health Sector Strategy on HIV 2016-2021. World Health Organization; 2016. (Accessed on Oct. 21, 2020)
   Available: http://www.who.int/hiv/strategy2016-2021/ghss-hiv/en/.
2. WHO. WHO - HIV vaccine development. WHO; 2015.
   Available:http://www.who.int/immunization/research/development/hiv_vaccdev/en/
   (Accessed on Oct. 21, 2020)
3. WHO. Global health sector strategy on HIV/AIDS 2011-2015. Strategy; 2011.
   DOI: 978 92 4 150165 1
4. UNAIDS, *Global Report 2012 with Annexes*. 2011.
5. CDC. Integrated guidance for developing epidemiologic profiles: HIV prevention and ryan white hiv/aids programs planning. 2014;1–132.
   Available:www.osti.gov/scitech/biblio/1222214-pore-size-distribution-methane-equilibrium-conditions-walker-ridge-block-northern-gulf-mexico%0Ahttp://www.cdc.gov/hiv/guidelines/%0Ahttp://hab.hrsa.gov/.
6. Mathers CD, Loncar D. Projections of global mortality and burden of disease from 2002 to 2030. PLoS Med. 2006;3(11):2011–2030.
   DOI: 10.1371/journal.pmed.0030442
7. McDonnel T. HIV Epidemic is mapped in sub-saharan africa to pinpoint hot spots : Goats and soda : NPR. www.npr.org; 2019.
   Available:https://www.npr.org/sections/goatsandsoda/2019/05/15/723564110/new-hiv-map-offers-most-detailed-look-yet-at-the-epidemic
   (Accessed on Oct. 21, 2020)
8. Vos T, et al. Global burden of 369 diseases and injuries in 204 countries and territories, 1990–2019: a systematic analysis for the Global Burden of Disease Study 2019. Lancet. 2020;396(10258)1204–1222.
   DOI: 10.1016/S0140-6736(20)30925-9
9. NACA. National HIV/AIDS strategic framework, 2017 - 2018. Natl. Agency Control AIDS; 2017.
   Available:https://www.childrenandaids.org/sites/default/files/2017-11/NATIONAL-HIV-AND-AIDS-STRATEGIC-FRAMEWORK.pdf.

10. Cichocki M. How HAART (Highly Active Antiretroviral Therapy) works; 2020. Available:https://www.verywellhealth.com/haart-highly-active-antiretroviral-therapy-48967 (Accessed Oct. 21, 2020)
11. Rodger AJ, et al. Risk of HIV transmission through condomless sex in serodifferent gay couples with the HIV-positive partner taking suppressive antiretroviral therapy (PARTNER): Final results of a multicentre, prospective, observational study. Lancet. 2019;393(10189):2428–2438. DOI: 10.1016/S0140-6736(19)30418-0.
12. Hammer SM, et al. A controlled trial of two nucleoside analogues plus indinavir in persons with human immunodeficiency virus infection and cd4 cell counts of 200 per cubic millimeter or less. N. Engl. J. Med. 1997;337(11):725–733. DOI: 10.1056/NEJM199709113371101
13. Arts EJ, Hazuda DJ. HIV-1 antiretroviral drug therapy. Cold Spring Harb. Perspect. Med. 2012;2(4):a007161. DOI: 10.1101/cshperspect.a007161
14. Kalyesubula R, et al. Hepatotoxicity from first line antiretroviral therapy: An experience from a resource limited setting. Afr. Health Sci. 2011;11(1):16–23. (Accessed on Oct. 21, 2020) Available:https://www.ncbi.nlm.nih.gov/pmc/articles/pmid/21572852/?tool=EBI
15. UNAIDS. Global HIV/AIDS overview | HIV.gov. Hiv.Gov; 2018. Available:https://www.hiv.gov/federal-response/pepfar-global-aids/global-hiv-aids-overview (Accessed on Oct. 21, 2020)
16. Mayo Clinic. Liver function tests - Mayo Clinic. Mayo Foundation for Medical Education and Research (MFMER); 2015. Available: https://www.mayoclinic.org/tests-procedures/liver-function-tests/about/pac-20394595 (Accessed on Oct. 21, 2020)
17. NIAID/NIH. Table for grading the severity of adult and pediatric adverse events. Natl. Inst. Allergy Infect. Dis. 2017;1–21. Available:https://rsc.niaid.nih.gov/sites/default/files/daidsgradingcorrectedv21.pdf
18. Kontorinis N, Dieterich D. Hepatotoxicity of antiretroviral therapy. AIDS Rev. 2003;5(1):36–43.
19. Llewellyn A, Simmonds M, Irving WL, Brunton G, Sowden AJ. Antiretroviral therapy and liver disease progression in HIV and hepatitis C co-infected patients: A systematic review and meta-analysis. Hepatol. Med. Policy. 2016;1(1). DOI: 10.1186/s41124-016-0015-7
20. Price JC, Thio CL. Liver disease in the hiv-infected individual. Clinical Gastroenterology and Hepatology. vol. 8, no. 12. Clin Gastroenterol Hepatol. 2010;1002–1012. DOI: 10.1016/j.cgh.2010.08.024
21. Ngala RA, Opoku D, Asare G. Effects of HIV infection and highly active antiretroviral therapy (HAART) on the liver of HIV patients. Trends Med. Res. 2015;10(1):1–11. DOI: 10.3923/tmr.2015.1.11
22. JR W, PE O. Anti-retroviral drug hepatotoxicity and risk factors in HIV patients with or without hepatitis B and C: A review. J. Infect. Dis. Ther. 2015;03(06)1–5. DOI: 10.4172/2332-0877.1000258
23. Neukam K, *et al.* "Liver toxicity of current antiretroviral regimens in HIV-infected patients with chronic viral hepatitis in a real-life setting: The Hepavir Seg-Hep Cohort. PLoS One. 2016;11(2):148104. DOI: 10.1371/journal.pone.0148104
24. Palella FJ, et al. Declining morbidity and mortality among patients with advanced human immunodeficiency virus infection. N. Engl. J. Med. 1998;338(13):853–860. DOI: 10.1056/NEJM199803263381301
25. Young B. Mixing new cocktails: Drug interactions in antiretroviral regimens. AIDS Patient Care and STDs. AIDS Patient Care STDS. 2005;19(5):286–297., DOI: 10.1089/apc.2005.19.286
26. Neuman MG, Schneider M, Nanau RM, Parry C. HIV-antiretroviral therapy induced liver, gastrointestinal, and pancreatic injury. Int. J. Hepatol. 2012;2012:1–23. DOI: 10.1155/2012/760706

27. Bonfanti P, et al. Risk factors for hepatotoxicity in patients treated with highly active antiretroviral therapy [2]. Journal of Acquired Immune Deficiency Syndromes. Lippincott Williams and Wilkins. 2001;27(3)316–318.
DOI: 10.1097/00126334-200107010-00017.
28. Sulkowski MS. Drug-Induced Liver Injury Associated with Antiretroviral Therapy that Includes HIV-1 Protease Inhibitors. Clin. Infect. Dis. 2004;38(Supplement_2):S90–S97.
DOI: 10.1086/381444
29. Mikl J, et al. Hepatic profile analyses of tipranavir in Phase II and III clinical trials. BMC Infect. Dis. 2009;9(203).
DOI: 10.1186/1471-2334-9-203
30. Núñez M, Soriano V. Hepatotoxicity of antiretrovirals: Incidence, mechanisms and management. Drug Safety. 2005;28(1):53–66.
DOI: 10.2165/00002018-200528010-00004
31. Monforte R, Antonella d'Arminio Bugarini, et al. Low frequency of severe hepatotoxicity and association with HCV coinfection in HIV-positive patients treated with HAART. JAIDS J. Acquir. Immune Defic. Syndr. 2001;28(2):114–123.
32. Pai VB, Koranyi K, Nahata MC. Acute hepatitis and bleeding possibly induced by zidovudine and ritonavir in an infant with HIV infection. Pharmacotherapy. 2000;20(9):1135–1140.
DOI: 10.1592/phco.20.13.1135.35024.
33. Sulkowski MS. Hepatotoxicity associated with antiretroviral therapy containing HIV-1 protease inhibitors. Semin. Liver Dis. 2003;23(2):183–193.
DOI: 10.1055/s-2003-39949
34. Flexner C. HIV-protease inhibitors. N. Engl. J. Med. 1998;338(18):1281–1293.
DOI: 10.1056/NEJM199804303381808
35. Núñez MJ, et al. Impact of antiretroviral treatment-related toxicities on hospital admissions in HIV-infected patients. AIDS Res. Hum. Retroviruses. 2006;22(9):825–829.
DOI: 10.1089/aid.2006.22.825
36. Soriano V, et al. Antiretroviral drugs and liver injury. AIDS. 2008;22(1):1–13.
DOI: 10.1097/QAD.0b013e3282f0e2fd
37. Mankhatitham W, Lueangniyomkul A, Manosuthi W. Hepatotoxicity in patients co-infected with tuberculosis and HIV-1 while receiving non-nucleoside reverse transcriptase inhibitor-based antiretroviral therapy and rifampicin-containing anti-tuberculosis regimen. Southeast Asian J. Trop. Med. Public Health. 2011;42(3):651–658.
38. Hernandez LV, Gilson I, Jacobson J, Affi A, Puetz TR, Dindzans VJ. Antiretroviral hepatotoxicity in human immunodeficiency virus-infected patients. Aliment. Pharmacol. Ther. 2001;15(10): 1627–1632.
DOI: 10.1046/j.1365-2036.2001.01086.x
39. Den Brinker M, et al. Hepatitis B and C virus co-infection and the risk for hepatotoxicity of highly active antiretroviral therapy in HIV-1 infection. AIDS. 2000;14(18):2895–2902.,
DOI: 10.1097/00002030-200012220-00011
40. Clark SJ, Creighton S, Portmann B, Taylor C, Wendon JA, Cramp ME. Acute liver failure associated with antiretroviral treatment for HIV: A report of six cases. J. Hepatol. 2002;36(2):295–301.
DOI: 10.1016/S0168-8278(01)00291-4
41. Kramer JR, Giordano TP, Souchek J, El-Serag HB. Hepatitis C coinfection increases the risk of fulminant hepatic failure in patients with HIV in the HAART era. J. Hepatol. 2005;42(3):309–314,
DOI: 10.1016/j.jhep.2004.11.017
42. Rodriguez-Rosado R, Garcia-Samaniego J, Soriano V. Hepatotoxicity after introduction of highly active antiretroviral therapy [8]. AIDS. 1998;12(10):1256.
DOI: 10.1097/00002030-199810000-00025
43. The Center for AIDS Information & Advocacy. High liver enzymes signal serious disease in HIV-Positive individuals without hepatitis; 2015.
Available:https://www.thebodypro.com/article/high-liver-enzymes-signal-serious-disease-in-hiv-p (Accessed on Oct. 21, 2020)
44. Mugusi S, et al. Liver enzyme abnormalities and associated risk factors in HIV patients on efavirenz-based HAART with or without tuberculosis co-infection in Tanzania. PLoS One. 2012;7(7):e40180.

DOI: 10.1371/journal.pone.0040180.
45. Cooper CL, Parbhakar MA, Angel JB. Hepatotoxicity associated with antiretroviral therapy containing dual versus single protease inhibitors in individuals coinfected with hepatitis C virus and human immunodeficiency virus. Clin. Infect. Dis. 2002;34(9):1259–1263.
DOI: 10.1086/339867
46. Heil EL, Townsend ML, Shipp K, Clarke A, Johnson MD. Incidence of severe hepatotoxicity related to antiretroviral therapy in HIV/HCV coinfected patients. AIDS Res. Treat. vol. 2010;856542–856542.
DOI: 10.1155/2010/856542
47. Unco JR, et al. Antituberculosis drug-induced hepatotoxicity: The role of hepatitis C virus and the human immunodeficiency virus. Am. J. Respir. Crit. Care Med., vol. 157, no. 6 PART I. 1998;1871–1876.
DOI: 10.1164/ajrccm.157.6.9711039
48. Jeong I, et al. Drug-induced hepatotoxicity of anti-tuberculosis drugs and their serum levels. J. Korean Med. Sci. 2015;30(2):167–172.
DOI: 10.3346/jkms.2015.30.2.167
49. Segamwenge IL, Bernard MK. Acute liver failure among patients on efavirenz-based antiretroviral therapy. Case Reports Hepatol. 2018;1–5.
DOI: 10.1155/2018/1270716.
50. Shiferaw MB, Tulu KT, Zegeye AM, Wubante AA. Liver enzymes abnormalities among highly active antiretroviral therapy experienced and haart naïve HIV-1 infected patients at debre tabor hospital, North West Ethiopia: A comparative cross-sectional study. AIDS Res. Treat; 2016.
DOI: 10.1155/2016/1985452
51. Tesfa E, Siefu D, Belayneh Y, Mekonnen Z. Liver enzyme elevation in patients taking HAART compared with treatment naïve controls at Debre Berhan Referral Hospital: A comparative cross-sectional study, Northeast Ethiopia. BMC Res. Notes. 2019;12(1).
DOI: 10.1186/s13104-019-4748-4
52. Sulkowski MS, Mehta SH, Chaisson RE, Thomas DL, Moore RD. Hepatotoxicity associated with protease inhibitor-based antiretroviral regimens with or without concurrent ritonavir. AIDS. 2004;18(17):2277–2284.
DOI: 10.1097/00002030-200411190-00008
53. Sterling RK, Chiu S, Snider K, Nixon D. The prevalence and risk factors for abnormal liver enzymes in HIV-Positive patients without Hepatitis B or C coinfections. Dig. Dis. Sci. 2008;53(5):1375–1382.
DOI: 10.1007/s10620-007-9999-6
54. Hooja S, Singhal A, Vyas N, Yadav R, Bachhiwal R. Hepatitis B virus seroprevalence and its correlation with CD4 cells and liver enzymes among human immunodeficiency virus positive individuals at a tertiary care hospital in North-West India. Int. J. Appl. Basic Med. Res. 2015;5(1):36.
DOI: 10.4103/2229-516x.149235
55. Murali AR. Liver test interpretation - Approach to the patient with liver disease: A guide to commonly used liver tests; 2017.
Available:http://www.clevelandclinicmeded.com/medicalpubs/diseasemanagement/hepatology/guide-to-common-liver-tests/#bib1
(Accessed Oct. 21, 2020)
56. Ruhl CE, Everhart JE. Upper limits of normal for alanine aminotransferase activity in the United States population. Hepatology. 2012;55(2):447–454.
DOI: 10.1002/hep.24725
57. Wu L, et al. Gender difference in the association between aminotransferase levels and hypertension in a Chinese elderly population. Med. (United States). 2017;96(21):e6996–e6996.
DOI: 10.1097/MD.0000000000006996.
58. Anyanwu CF, Obianime WA, Siminialayi IM, Aigbogun EO. Evaluating the effects of Moringa oleifera on atherogenic lipoprotein indices of HIV infected Nigerian adults on tenofovir-based antiretroviral regimen. Int. J. Basic Clin. Pharmacol. 2019;8(6):1312.
DOI: 10.18203/2319-2003.ijbcp20192195
59. Kim IH, Kisseleva T, Brenner DA. Aging and liver disease. Current opinion in gastroenterology. Lippincott Williams and Wilkins. 2015;31(3):184–191.

DOI: 10.1097/MOG.0000000000000176
60. Koehler EM, et al. Prevalence and risk factors of non-alcoholic fatty liver disease in the elderly: Results from the Rotterdam study. J. Hepatol. 2012;57(6):1305–1311.
DOI: 10.1016/j.jhep.2012.07.028
61. Anyanwu CF, Jr. EOA, Joseph TO. Evaluation of the liver enzyme (AST, ALT & ALP) levels of adult HIV patients on HAART in UPTH. Annu. Res. Rev. Biol. 2020;35(3):34–41.
DOI: 10.9734/arrb/2020/v35i330198
62. Biosciences B. BD FACScalibur flow cytometer the automated, multicolor flow cytometry system. 2002;1–11.
Available: http://med.fau.edu/calibur.pdf
63. CDC. Guidelines for the performance of CD4+ T-cell determinations in persons with human immunodeficiency virus infection. MMWR. Recomm. Rep. 1992;41(RR-8):1–17.
(Accessed on Oct. 23, 2020)
Available:https://www.cdc.gov/mmwr/preview/mmwrhtml/00019952.htm
64. Maida I, et al. Severe liver disease associated with prolonged exposure to antiretroviral drugs. JAIDS J. Acquir. Immune Defic. Syndr. 2006;42(2):177–182.
DOI: 10.1097/01.qai.0000221683.44940.62
65. Saag MS, et al. Antiretroviral drugs for treatment and prevention of HIV infection in adults: 2018 recommendations of the international antiviral society-USA panel. in JAMA - Journal of the American Medical Association. 2018;320(4):379–396,
DOI: 10.1001/jama.2018.8431
66. Thurman AR, Anderson S, Doncel GF. Effects of hormonal contraception on antiretroviral drug metabolism, pharmacokinetics and pharmacodynamics. Am. J. Reprod. Immunol. 2014;71(6): 523–530.
DOI: 10.1111/aji.12210
67. Wit FWNM, Weverling GJ, Weel J, Jurriaans S, Lange JMA. Incidence of and risk factors for severe hepatotoxicity associated with antiretroviral combination therapy. J. Infect. Dis. 2002;186(1):23–31.
DOI: 10.1086/341084
68. Selvaraj S, et al. Antiretroviral therapy-induced mitochondrial toxicity: Potential mechanisms beyond polymerase-γ inhibition. Clin. Pharmacol. Ther. 2014;96(1):110–120.
DOI: 10.1038/clpt.2014.64
69. Apostolova N, Gomez-Sucerquia LJ, Moran A, Alvarez A, Blas-Garcia A, Esplugues JV. Enhanced oxidative stress and increased mitochondrial mass during Efavirenz-induced apoptosis in human hepatic cells. Br. J. Pharmacol. 2010;160:2069–2084.
DOI: 10.1111/j.1476-5381.2010.00866.x
70. Stein JH. Dyslipidemia in the era of HIV protease inhibitors. Prog. Cardiovasc. Dis. vol. 2003;45(4):293–304.
DOI: 10.1053/pcad.2003.3
71. Mulligan K, et al. Hyperlipidemia and insulin resistance are induced by protease inhibitors independent of changes in body composition in patients with HIV infection. JAIDS J. Acquir. Immune Defic. Syndr. 2000;23(1):35–43.
DOI: 10.1097/00126334-200001010-00005
72. Hui DY. Effects of HIV protease inhibitor therapy on lipid metabolism. Progress in Lipid Research. 2003;42(2):81–92.
DOI: 10.1016/S0163-7827(02)00046-2
73. Powderly WG, Carr A. Clinical treatment. AIDS. 2001;15(SUPPL. 5).
DOI: 10.1097/00002030-200100005-00019
74. Emejulu AA, Ujowundu CO, Igwe CU, Ouwuliri VA. Hepatotoxicity of antiretroviral drugs in HIV seropositive Nigerian patients. Aust. J. Basic Appl. Sci. 2010;4(9):4275–4278.
75. Eluwa GI, Badru T, Akpoigbe KJ. Adverse drug reactions to antiretroviral therapy (ARVs): Incidence, type and risk factors in Nigeria. BMC Clin. Pharmacol. 2012;12(7).
DOI: 10.1186/1472-6904-12-7
76. Snežana B. The Declaration of helsinki - The cornerstone of research ethics. Arch. Oncol. 2001;9.

Biography of author(s)



Dr. Chinwe F. Anyanwu
Department of Pharmacology, Faculty of Basic Clinical Sciences, University of Port Harcourt, Nigeria.

Research and Academic Experience: 3 years

Research Area: Immunopharmacology and Reproductive toxicity

Number of Published papers: 2



Dr. Eric O. Aigbogun Jr.
Department of Anatomy, Faculty of Medical Sciences, Kampala International University, Western Campus, Uganda.

Research and Academic Experience: 8 years

Research Area: Data Science and Research Design

Number of Published papers: 52

Special Award: Nominated for Fellow of Institute of Management Consultants (FIMCN), Nigeria

© Copyright (2021): Author(s). The licensee is the publisher (Book Publisher International).

DISCLAIMER
This chapter is an extended version of the article published by the same author(s) in the following journal.
Annual Research & Review in Biology, 35(3): 34-41, 2020.

Reviewers' Information
(1) Abayomi Afe, Texila American University, Guyana.
(2) Idorenyin Umoh, University of Uyo, Nigeria.

Chapter 5
Print ISBN: 978-93-90516-93-3, eBook ISBN: 978-93-90516-94-0

An Overview of a Neurocosmological Neurotheology Organized around Reissner's Fiber

L. Wile[1*]

DOI: 10.9734/bpi/rrab/v5/7359D

ABSTRACT

The subjects of neurotheological investigations have thus far made dubious claims that their experiences are equivalent to the purported revelatory and mystical experiences of the originators of the major religions and mystical traditions. Parallels between ancient mysticism and modern physics have thus far mostly dissolved into poetic metaphors. However, a new approach to testing the ancient claims of supersensory perceptions of absolute truth is on the horizon. Mystical traditions have consistently described a circuit running through the center of the spine that connects the human and the divine. If descriptions of this circuit are based on actual interoceptions, then it corresponds to a little-known, epigenetically suppressed structure that ensheathes the central axis of the central nervous system; Reissner's fiber (RF). A regenerated fiber could create a new relationship with realities that quantum orthodoxy has declared to be beyond conception or perception, a relationship that could to lead to a marriage between the origins of religion and the frontiers of science.

Keywords: Neurotheology; quantum biology; subtle anatomy; quantum mysticism.

ABBREVIATIONS

RF : Reissner's Fiber;
AUB : Absolute Unitary Being;
CSF : Cerebrospinal fluid

1. INTRODUCTION

Mystical experiences, the experiential basis of religion, are purported supersensory perceptions of absolute truth. A critical problem for neuroethology's search for the neural correlates of such experiences is that its subjects have only their subjective experiences to justify their status as mystics.

During the 20th century, physicists discovered what is deemed to be a final limit to the apprehension of absolute truth.: The nature of quanta is beyond any perception or conception. Regarding this limit of objective knowledge, Werner Heisenberg stated, "There is no use in discussing what could be done if we were other beings than we are [1]."

However, the originators of the earliest mystical traditions not only claimed to have apprehended ultimate reality-- referred to by yogis, Taoists and kabbalists as Brahman, Tao, and Ein-Sof, respectively--but they also described a "subtle anatomy" organized around the central axis of the central nervous system. When activated by various practices, the "subtle anatomy" makes us beings other than we are.

If the "subtle anatomy" is real, then its central feature corresponds to a little-known, enigmatic, epigenetically suppressed structure that unsheathes the central axis of the central nervous system; RF [2,3]. Unlike the current methodology of neurotheology, which investigates the neural correlates of

[1]*Chaikin-Wile Foundation, 8 Winged Foot Blvd., Bloomfield CT, 06002, USA.*
**Corresponding author: E-mail: chaikinwile@aol.com;*

known varieties of religious experiences, a neurotheology organized around RF would regenerate a unique, spectacularly strategically located lost neural circuit and search for supersensory experiences beyond the capabilities of our current neural wiring. Individuals who can transcend quantum uncertainty would have an objective basis to justify their status as mystics.

2. CURRENT NEUROTHEOLOGICAL SUBJECTS AND THEIR LIMITATIONS

The blissful unity resulting from the dissolution of the self and external reality achieved by meditators has been proposed to be the realization of mysticism. Newberg, for example, dubbed this experience "Absolute Unitary Being" (AUB) and argued that the unification between the knower and the known does not allow "competing version of the truth; there is only truth itself [4]." However, AUB nullifies the intellect and deafferentiates sensory input rather than transcend them. AUB is a regression to oceanic feelings prior to the separation and individuation of the ego rather than transcendence of the ego. AUB is a subsensory experience devoid of phenomenological content not a supersensory perception of absolute truth.

Individuals under the influence of psychedelics have also been the subjects of neurotheological investigations. They report that logical chains and social conditioning that shape our perceptions dissolve. Aldous Huxley exulted after opening the "doors of perception" with mescaline, "I was seeing what Adam had seen on the morning of his creation – the miracle, moment by moment, of naked existence [5]." The neurophysiology underlying the altered perceptions produced by psychedelics provide insights into Huxley's assessment. Under the influence of lysergic acid diethylamide, (LSD), neuronal firing self-organizes spontaneously at a balance between order and chaos [6]. Neural activity thereby attunes itself to "naked existence," "moment to moment" instead of constructing reality through inflexible concepts. However, like meditators, psychedelic users show no evidence of transcending the current limits of objective knowledge. Nevertheless, the ability of meditation and psychedelics to dissolve the concepts that constitute our egos and structure our perceptions of reality can be used as a tool to explore human consciousness.

3. DEVIL ACCORDING TO BAUDELAIRE

Although RF has been conserved along all the branches of the 550-million-year-old chordate phylogenetic tree from amphioxus to *homo sapiens* and occupies the most strategic location in the central nervous system, it has been neglected by neuroscientists. Two historians of neuroscience recently dubbed RF the "Devil according to Baudelaire" whose "loveliest trick" is convincing us that he doesn't exist [7].

For nearly fifty years after its discovery by Ernst Reissner [8] in 1860, in spite of the fact that he distinguished "this cord from axons or irregular masses previously observed floating in the central canal," neuroscientists continued to mistakenly refer to it as an axon, cellular debris or an artifact of preservation [9]. Porter Sargent, who observed the fiber 1899 while dissecting fish, was astonished that "so peculiar and conspicuous a structure as RF, which is of so great importance in the nervous anatomy as to persist throughout the vertebrate series, should have remained for forty years after its discovery so little known [10]." His neuroanatomical studies and behavioral experiments convinced him that the fiber is a novel, specialized pathway for the high-speed transmission of signals that mediate an "optic reflex apparatus" for the "short circuit transmission of motor reflexes."

Sargent concluded his 1905 paper by saying, "The conclusions and the discussion of the results and bearings of this research are reserved for the second part of this paper dealing with the higher vertebrates. This is already well advanced, and it is hoped will appear in about a year." However, Sargent never published the second part of his paper. He abruptly abandoned his academic career and spent the next decade traveling the globe, "re-educating myself and others, studying and interpreting peoples, their arts and religions."

For several years, Sargent's hypothesis that RF is a novel pathway for the high-speed transmission of signals was generally accepted. In 1908, giving "greatest deference" to Sargent's opinions regarding the fiber's function, pioneering neurosurgeon Sir Victor Horsley undertook an investigation of RF in

apes [11]. Owing to the fragility of the fiber and its elastic recoil, Horsley was able to examine it adequately in sagittal sections of the spinal cord in only one specimen of *Maccacus cynomolgus.* He showed that the fiber lacked the structure of nerves and did not display Wallerian degeneration. He urged reinvestigation of the fiber before dismissing it as vestigial.

While Sargent distinguished the "axons" that form RF from "ordinary axis cylinders," described the fiber as a "highly specialized conduction path," and contrasted the "very thin medullary sheath" surrounding the fiber with the sheath surrounding "ordinary nerves," he nevertheless labeled the fiber as a coalescence of axons from various nuclei that project into the ventricles. Rudolph Albert von Kölliker, who, in 1898, coined the term axon, was more cautious. He was unable to decide if RF was an axon, an artifact of preservation, or a "crystallization of biological secretions [12]."Electron microscopic studies show that the fiber is formed by the aggregation of glycoproteins secreted primarily by the subcommissural organ [13].

The realization that the fiber is not an axon led to the rejection of Sargent's hypothesis that the fiber is a novel, specialized pathway for the high-speed transmission of signals.

In 1907 Dendy and Nicholls proposed that the fiber is an elastic cord. Varying tensions of the fiber resulting from movements of the body are relayed to sensory cells in the subcommissural organ which in turn regulate those movements [14]. As the conceptualization of RF transformed from a novel high-speed transmitter of signals associated with the visual system to an elastic cord, it became invisible to neuroscientists. Since new facts have come to light, the fiber has been hypothesized to play a role in the pathogenesis of hydrocephalus [15] and the "detoxification" of the cerebrospinal fluid (CSF) [16]. Overshadowed by neuronal physiology, however, the fiber continues to be neglected.

Further contributing to the fiber's obscurity is the fact that the scientific literature that does refer to the fiber usually states that it does not exist in humans [17]. However, the fiber is active during human embryological development, influencing neurogenesis along the adjacent ependymal border and guiding neuronal migration and synaptogenesis via the extracellular matrix [18,19]. Also, while the fiber typically undergoes regression during the last trimester of fetal development, it has been observed in a 14-year-old teenager [20]. The subcommissural organ typically regresses during infancy, but a fully developed organ has been observed in a 60-year-old man [21]. The central canal typically occludes during childhood, but sometime persists into adulthood. Surprisingly, the occlusion is not the result of age-dependent degeneration, but of inflammation of the canal's ependymal lining [22].

RF undergoes rapid post-mortem degeneration and soon becomes indistinguishable from the ground substance and cilia along the ventricular lining to which it adheres. Other rare anomalies, therefore, might have gone undetected. The words of the French investigator of RF, Etienne-Jules Legait [23] remain true today: "when its existence is denied, this fact should be carefully analyzed and discussed: one could not take it into account if fixation is uncertain. "Current neuroimaging using magnetic resonance imaging or positron emission tomography lack sufficient resolution to detect the fiber in living subjects.

4. CIRCUMNEUROCELIC SENSORY SYSTEM

RF's remarkably strategic location first manifests itself during embryological development, leaving its imprint on each of the neural stem cells along the ventricular lining. In the adult, RF is surrounded by a circumneurocelic sensory system (CNSS) along the walls of the third and fourth ventricles, central canal and terminal ventricle. While investigations of the sensory role of CSF-contacting neurons and circumventricular organs are still in their early stages [24,25], similarities between the rods and cones of the retina and the hair cells of the inner ear, and the cilia lining the neurocele, prompted speculations that it might be an interoceptive sensory system more than 100 years ago. In 1913, Dimitri Tretjakov dubbed this system the "central sense organ [26]." William Kolmer later elaborated on Tretjakov's proposal, comparing RF to the gel- like tectorial membrane of the inner ear which lies beneath Reissner's membrane. He named this novel sensory system the "sagittal organ [27]."

CSF-contacting neurons from the dorsal raphe nucleus, the site of action of LSD [26], the locus coeruleus, which innervates extensive areas of the brain [28], and opiate receptors surrounding the periaqueductal gray area [29] contact filaments projecting from RF [30]. Some CSF-contacting neurons are photoreceptors [31]. Afferent pathways from the CNSS could have generated interoceptions of the "subtle anatomy."

5. EVOLUTION

While the unique capacity of *Homo sapiens* to create an ever-expanding world of knowledge that liberates us from genetically programmed behaviors and even allows us to engineer our own evolution suggests an evolutionary leap, the neural correlates of that leap are unclear. Einstein [32] proposed that humankind's first cognitive leap was the commission of the "metaphysical original sin," the creation of the concept of physical objects independent of our stream of subjective sensations. The differentiation between sense impressions and "mere ideas," upon which the positing of physical reality is based, cannot be logically justified, hence Einstein's characterization of the creation of the concept of independently existing physical objects as a "sin." Which, in Einstein's opinion, is necessary to avoid solipsism.

The road forward from the "metaphysical original sin," required a leap from an analog to a digitized system of language around 100,000 years ago. Noam Chomsky proposes that this evolutionary leap resulted from a chance mutation that produced the neuroanatomical basis of language in "near perfect form, "planting a seed crystal in a supersaturated solution to create a digital infinity [33]. Given the fiber's remarkably strategic location, the possibility that its current typical fetal regression mirrors its recent epigenetically induced evolutionary regression is an attractive alternative to the hypothesis that a chance mutation "planted a seed crystal" that created the digital infinity of language. Analyses of human epigenomes from the era in question could test this hypothesis.

Science developed from the desire for logical unification of "primary concepts," concepts directly connected with sensory experience and propositions connecting them. Scientifically minded individuals created increasingly more unified systems from which this primary layer of complexes of sensory experience could be logically derived. The price paid for this logical unity is that the deeper levels are increasingly removed from sensory experience. The originators of the earliest mystical traditions claim to have directly experienced ultimate unity beyond logic and sensory experience.

According to the theory proposed here, the originators of mystical traditions resisted the epigenetic suppression of RF. They learned digitized language and "heard" and "saw" the input from the fiber. They cognized deeper levels of language wherein the vibrations of spoken words and the geometry of written words were not merely arbitrary sounds and signs arbitrarily associated with sensations but were intrinsically connected to subtle levels of reality [34].

Standing before ancient Sanskrit, Chinese, and Hebrew scriptures without RF, we are like aphasics lacking Wernicke's area. Not only have we lost the ability to comprehend the mystical levels of language, but without RF, we no longer perceive the realities to which they refer. Our reality is in an interpretation of neural activity. RF's stimulation of the CNSS would have been commensurate with the stimulation of our external sensory organs and could have thereby generated an alternate perception of reality.

6. REISSNER'S FIBER AND THE "SUBTLE ANATOMY"

The notion that ancient mystics interocepted subtle neurophysiological processes responsible for supersensory perceptions that are just now becoming accessible to modern technology, has been almost universally dismissed by neuroscientists. The identifications of RF with the central axis of the "subtle anatomy" has been overshadowed by popularized accounts of the "subtle anatomy" based on loose associations with neural plexuses, glands, organs and genitalia, and obscured by the fiber's role as the "Devil according Baudelaire." Nevertheless, in 1940, Theos Bernard [35], after meeting with yogis, scientists and scholars in India, wrote in his book *Heaven Lies Within Us,* "Inside this central (*Sushumna*) nadi, the Yogi identifies an invisible nadi known in the West as the fibre of Reissner, but

which is known here as *Chittra* (the Heavenly Passage, in Sanskrit)." His identification was based largely on Sir John Woodroffe's [36] translation of the Shatchakra *Nirupana* ("Description of and Investigation into the Six Bodily Centres") from its original Sanskrit. The text describes three concentric "canals" within the *Sushumna nadi,* the *Vajra. Chittra* and *Brahma nadis.* The second verse states, "Inside the *Vajra* is *Chittra . . .* She is subtle as a spider's thread." Inside the *Chittra nadi* is the innermost *nadi¸* the *Brahma nadi* whose entrance is described in the forty-eighth verse, as "extremely subtle and like unto the ten-millionth part of the end of a hair." The later discovery that the several micron diameter fiber is hollow is consistent with the identification of the *Brahma nadi* with the canal running through the fiber [37]. However, RF as the *Chittra nadi* became the Devil according to Baudelaire disguised as a yogi peddling occult dreams.

In 1973, as a second-year medical student, I rediscovered the identification of RF with the innermost *nadis.* The fiber had played it devilish trick so well, that it was only after 18 months of an intensive investigation of the neurocele, under the guidance of Dr. Charles Loeser, Chairman of the Neuroscience Department, University of Connecticut School of Medicine, that I discovered the fiber by a fortuitous encounter with an article titled, "Studies concerning the function of the complex subcommissural organ-liquor fibre [38]." It was not until 40 years later, following years of computer searches using various combinations of keywords coupled with RF that I learned of Bernard's earlier identification of the *Chittra nadi* with the fiber.

Shortly before our discovery of the fiber, while searching for the anatomical equivalent of the *Mooladara chakra*--a triangular region at the base of the spinal cord where the *Chittra nadi* coils--we had observed previously unreported circular clusters of cells surrounding the triangular terminal ventricle which are morphologically similar to secretory cells. These structures were later rediscovered and named the "intraspinal organ [39]." The function of the terminal ventricle remains unknown. RF coils in the piscine equivalent of the terminal ventricle [40], the urophysis which secretes the hormone urotensin.

The "subtle anatomy" underlying Taoist mystical traditions, especially the governing and conception vessels that serve as central reservoirs of *Qi,* closely corresponds with the central *nadis* of yoga. Like the yogic anatomy, the anatomy underlying mystical Taoist practices such as Qi Gong and acupuncture as applied Taoism is generally dismissed as a misinterpretation of poetic metaphors based on primitive anatomical knowledge. However, recent investigations of the anatomical basis of acupuncture provide evidence that the governing vessel corresponds to RF.

In 1960, a North Korean scientist named Kim Bonghan [41] injected radioactive phosphorous (P^{32}) into acupuncture points on a rabbit's abdomen. He found that injections into these points followed meridians along a novel network of ducts. Injections into other sites dispersed. Injections into the acupuncture point corresponding to the Governing Vessel labelled a thread-like structure inside the central canal. Bonghan made no reference to RF and named the labelled structure the "neural Bonghan duct."

Bonghan was praised by the North Korean government for discovering a "monumental theory in global science" and elevated to a high position in the government. However, amidst political intrigue and allegations that he'd withheld details of his methods he was discredited and banished. The scientific community dismissed Bonghan's findings for several decades. In 2005, neuroscientists "rediscovered" the network of Bonghan ducts and proposed that they act as optical channels for coherent biophotons [42]. In 2008, his team reported the discovery of a novel thread-like structure in the cerebral ventricles and central canal in a rabbit [43]. They claimed the "novel thread-like structure" they had observed was different from RF yet occupies the same space!

The Kabbalistic counterpart to the central *nadis* and the governing vessel is the central pillar of the *Sephirot*, the symbolic representation of the divine image in which man was created. Wary that anatomical descriptions of the human microcosm could lead to anthropomorphic conceptions of God, Kabbalists have traditionally interpreted the *Sephirot* in abstract psychological and spiritual terms. Nevertheless, Charles Ponce] has proposed that the *Sephirot* represent a lost form of yoga [44]. His explanation of Kabbalah provides an unintended poetic allusion to the fiber:

> There's an Adam within each of us…in exile from the Garden. The aim of Kabbalah is the restoration of the divine man in the medium of mortal man…We are the laboratory…If one can learn to connect the thread dangling free from the *Sephirot* with the thread of one's own being, one may begin the work of restoration.

Unlike the mystical traditions of India and China which developed yoga, acupuncture and Qi Gong, the Hebrew tradition focused on the moral laws of the Torah. Its anatomical secrets were lost. However, the miraculous fasts of 40 days and 40 nights by three key Biblical figures, Moses, Elijah and Jesus, who appeared during the Transfiguration of Jesus, is consistent with a functioning RF. The fiber's role in regulating salt and water balance, blood pressure and the biological clock could have averted electrolyte imbalances, hypovolemic shock and produced a state of suspended animation.

If near-death experiences are evidence of disembodied, mystical experiences, then perceptions of the "silver cord" might be a variety of an interoception of the "subtle anatomy." As the brain's energy drains centripetally toward its phylogenetically ancient core, the CNSS might generate a percept of a virtual RF [45].

7. REISSNER'S FIBER AND QUANTUM BIOPHYSICS

Ever since physicists saw that the equations of quantum mechanics point to an indistinct, unvisualizable, immaterial reality that mysteriously transitions to a distinct physical reality, they have pondered the relationship between the unity of consciousness and the cosmos described by ancient mystics, and the ambiguous reality they had encountered. Wolfgang Pauli called for "a synthesis embracing both rational understanding and the mystical experience of unity," that would be achieved when "natural sciences will out of themselves bring forth a counter pole in their adherents, which connects to the old mystic elements [46]."

Erwin Schrodinger recommended, "a bit of a blood-transfusion from Eastern thought," but warned, "we must beware of blunders – blood transfusion always needs great precaution to prevent clotting. We do not wish to lose the logical precision that our scientific thought has reached . . . [47]."

By the 1950s, however, quantum orthodoxy rejected mysticism [48]. While mystics claim that supersensory perceptions of absolute truth are possible, physicists hold that we must be content with "mysteries without mysticism [49]." We are, under currently known circumstances, denied perception of quanta. Somewhere along the pathway to the occipital cortex that subserves visual perceptions, quanta probabilistically transition to classical realities. However, RF might have provided "immaculate perceptions" of the *paranormal quantum world whose descriptions are buried under the dust of fallen Babel.*

Weighing against this hypothesis is the long held conventional wisdom that quantum effects are negligible in the warm noisy brain. Macroscopic systems were long thought to exist only at temperatures near absolute zero such the Bose-Einstein condensate that was produced in a vapor of rubidium atoms that was cooled to 170 nanokelvin in 1995. However, the recent discoveries of quantum coherences in photosynthetic systems [50], the retina [51], the avian compass [52], and new understandings of quantum feedback and control, suggest that non-trivial quantum effects in the brain are possible [53,54]. RF is uniquely well-suited to implement the principles of quantum entanglement, coherence and information processing that are currently beings harnessed by quantum technologies. Biophotons emitted by the fiber could stimulate photoreceptors of the CNSS whose signals would be analyzed by the brain which would send biophotonic signals to promote quantum coherences in a manner analogous to quantum cavity electrodynamic systems. Such promotion of quantum coherences could arise spontaneously from dissipative processes and enhanced by biofeedback

But even if it is possible to directly perceive and control RF as a macroscopic quantum system, is there justification for the claim that such perceptions of reality beyond the limits of quantum uncertainty are the purported supersensory perceptions of the originators of the earliest mystical traditions? Does more precise measurement and control of atomic phenomena in RF translate into the blessedness, freedom, and love that mystics claim is entailed by supersensory percepts of absolute truth. The proof is in the pudding.

8. THE ROAD TOWARD ANEUROCOSMOLOGICAL NEUROTHEOLOGY ORGANIZED AROUND REISSNER'S FIBER

The road toward testing the hypothesis that RF is the "subtle anatomy" described by mystical traditions is long and difficult and travels along several converging paths. One hundred and twenty years after Sargent expressed his astonishment that such an evolutionarily conserved strategically located structure had been neglected, the fiber still plays the Devil's loveliest trick of convincing neuroscientists that it doesn't exist.

However, as quantum biophysical models of consciousness challenge the existing materialist paradigm that understands consciousness as an emergent property of networks of binary neural spikes, RF is emerging from the shadows. If consciousness is fundamental and coeternal with quantum fields, the fiber's 5-nanometer filaments are uniquely well-suited to serve as an interface between those fields and neural events.

Twenty years ago, my team of scientists led by Professor Vasili Kharchenko, Harvard-Smithsonian Center for Astrophysics and Harvard Physics Department, and Professor Alexander Sergienko, Department of Physics, Boston University Photonics Center, began searching for quantum coherences in the fiber. We have developed a novel microspectrometer to analyze biophotonic emissions from the fiber in living zebrafish larvae and similarly transparent adult zebrafish mutants (Caspar line) using the technology of correlated photon counting with superconducting single-photon detectors coupled to a time-correlated counting system with picosecond resolution. Our apparatus is equipped with femtosecond pulsed infrared lasers to perform photon echo-based experiments similar to those recently used to reveal the quantum mechanisms involved in light harvesting during photosynthesis. We have also begun searching for electron spin coherences using nitrogen vacancy diamonds. Thus far, the signal-to-noise ratio has been too low to draw meaningful conclusions.

In addition to collecting data, we are developing mathematical models based on string theories. Because strings operate near Planck units of length, (10^{-35} meters), energies required to collect data astronomically exceed the most power particle accelerators. Because they interact with branes of higher dimensions in a in a cosmic landscape of 10^{500} parallel universes, they can accommodate almost any prediction. A condensate of strings running through the hollow core of RF could amplify the effects of strings to detectable levels. The ultimate identification of RF with the central axis of the "subtle anatomy" would be a translation of mysticism's core belief that we are a microcosm of the macrocosm into a neurocosmology in which the 1-dimensional thread running through the center of the fiber is commensurate with the initial cosmic singularity and the cosmos by applying holographic principles [55].

My team has also developed a transgenic zebrafish in which the expression of green fluorescent protein is under control of the promoter for F-spondin. F-spondin, a protein associated with the fiber, play a key role in neurogenesis [56] and circadian rhythms [57]. It has been implicated in the pathogenesis of Alzheimer's disease because overexpression of f-spondin improves memory and reduces amyloid-β levels in mice [58]. We are also using confocal imaging techniques to better understand the terminal ventricle's glandular function.

9. CONCLUSION

RF eluded observation for thousands of years. During the 20th century, after a brief interlude in the spotlight, it played its role as the Devil according to Baudelaire. Now the fiber is poised to reveal its secrets. The possibility that interoceptions and neuroimaging of RF can achieve a synthesis of empirical science and the original mystical experiences of unity is becoming a testable hypothesis as a neurocosmological neurotheology. The circumstantial evidence supporting the hypothesis is intriguing, but, thus far, insufficient to persuade the skeptic.

COMPETING INTERESTS

Author has declared that no competing interests exist.

REFERENCES

1. Heisenberg W, quoted in: Herbert N. Quantum Reality: Beyond the New Physics, Garden City, New York: Anchor Press/Doubleday; 1985.
2. Wile L. Reissner's Fibre: A forgotten Pathway for Exploring Consciousness, Journal of Consciousness Studies. 2016;23:191-211.
3. Wile L. The Jaynes Legacy" Shining New Light Through the Cracks in the Bicameral Mind, Exeter, UK: Imprint Academic; 2018.
4. Newberg A. et al. Why God Won't Go Away: Brain Science and the Biology of Belief.New York: Ballantine Books; 2001.
5. Huxley A. The Doors of Perception: and Heaven and Hell. New York: Harper& Row; 1963.
6. Harris R et al. Neural correlates of the LSD experience revealed by multimodal neuroimaging. PNAS; 2016. DOI.ORG/10.1073/PNAS.1518377113
7. Olry R, Haines D. Reissner's Fiber: The exception which proves the rule, or the Devil according to Charles Baudelaire? Journal of the History of the Neurosciences. 2003;12:73-75.
8. Reissner E. Beitragezur KenntissvomBau des Ruskenmarks von Petromyzon fluvatis, Arch. Anat. Physiol. 1860;445-488.
9. Nicholls D. The Structure and Development of Reissner's Fibre and the Sub-commissural Organ; 1912.
   Available:http://jcs.biologists.org/content/joces/s2-58/229/1.full.pdf
10. Sargent PE. The optic reflex apparatus of vertebrates for short-circuit transmission of motor reflexes through Reissner's fiber: Its morphology, ontogeny, phylogeny and function. Part I. The fish-like vertebrates, Bulletin of the Museum of Comparative Zoology at Harvard University. 1905;45:1–256.
11. Horsley V. Note on the existence of Reissner's fiber in higher vertebrates, Brain. 1908;31:147-59.
12. Kolliker A. Uber die oberflachichen Nervenkerneim Marke der Vogel un Reptilien, Zeitschr. F. will. Zool, Bd; 1902.
13. Weindl A, Schinko L. Evidence by scanning electron microscopy for ependymal secretion into the cerebrospinal fluid and formation of Reissner's fiber by the subcommissural organ. Brain Research. 1975;88:319–324.
14. Dendy A, Nicholls D. On the occurrence of a mesocoelic recess in the human brain and its relation to the subcommissural organ in lower vertebrates; with special reference to the distribution of Reissner's fiber in the vertebrate series and its possible function. Anat. Anz. 1910;37:496–508.
15. Woolam DHM. The circumventricular organs of the brain: Their possible role as sites for future neurosurgery. Annals of the Royal College of Surgeons of England. 1982;64:310–317.
16. Diederen JH, et al. The subcommissural organ-liquor fibre complex: the binding of catecholamines to the liquor fibre in frogs of the Rana esculenta complex. Acta Zool (Stockh). 1983;64:47–53.
17. Galarza M. Evidence of the subcommissural organ in humans and its association with hydrocephalus, Neurosurgical Review. 2002;25:205–215.
18. Monnerie H, et al. Reissner's fiber promotes neural aggregation and influences neuritic outgrowth in vitro.Cell and Tissue Research. 1997;287:285–295.
19. Rodriguez EM, et al. Human subcommissural organ, with particular emphasis on its secretory activity during the fetal life Microscopy Research and Technique.
   Doi.Org/10.1002/1097-0029(20010301)52:5<573::Aid-Jemt1042>3.0.Co;2-6.
20. Agduhr E. Uber einzentrales Sinnesorgan (?) bei den Vertebraten. Zischr. Anat. Entwickelungesch. 1922;66:223-30.
21. Gomez B, Benito-Aaranz E, Rodriguez M. The subcommissural organ in the grown-up man.Acta Anatomica. 1961;44:98–103.
22. Saker F. The Human Central Canal of the Spinal Cord: A Comprehensive Review of its Anatomy, Embryology, Molecular Development, Variants, and Pathology. Cureus; 2016.
   DOI: 10.7759/cureus.927
23. Legait G. Les Organes epenymaires du troisemeventricle. L'organesou-commissural. Lorgane sub-fornical. L'organe para-ventriculare. Nancy, Georges Thomas. 1942;72-104.

24. Vigh B et. al. The system of cerebrospinal fluid-contacting neurons. Its supposed role in the nonsynaptic signal transmission of the brain.Histol. Histopathol; 2004.
DOI: 10.14670/HH-19.607
25. Gross P, Weindl A, Knigge K. Adolf, Karl M. Peering through the Windows of the Brain.Journal of Cerebral Blood Flow & Metabolism; 1987.
DOI.ORG/10.1038/JCBFM.1987.120
26. Tretjakov D. Die zentralen Sinnesorgane be iPetromyzon,.Arch f. mikr. Anat. 1913;53.
27. Kolmer W. Uher das Sagittalorganein ventricles Sinneorgan des Wirheltiereins besonderebein Affen. Zeitschrift Zellforsch. 1931;13:1236-1248.
28. Feng C-Y, et al. The Locus Ceruleus Responds to Signaling Molecules Obtained from the CSF by Transfer through Tanycytes**.** J. Neurosci; 2011.
DOI: 10.1523/JNEUROSCI.5018-10.2011
29. Veening JG. Volume transmission of beta-endorphin via the cerebrospinal fluid; a review. Fluids Barriers CNS. 2012;9(1):16.
DOI: 10.1186/2045-8118-9-16
30. Kohno K. Electron microscope study of Reissner's fiber, Z. Zellforsch. 1969;94:565-573.
31. Fernandes AM, Fero K. Enlightening the brain: Linking deep brain photoreception with behavior and physiology. Current Biology. 2013;22:2042–2047.
32. Einstein A. Ideas and Opinions. New York: Crown Publishers; 1953.
33. Chomsky N. Linguistics and Cognitive Science: Problems and Mysteries. in Asa Kasher (ed.).The Chomskyan Turn. Oxford: Blackwell. 1991;26-53.
34. Holdrege A. Veda and Torah: Transcending the Textuality of Scripture. Albany: State University of New York Press; 1996.
35. Bernard T. Heaven Lies Within Us, New York: Rider and Company; 1940.
36. Woodroffe, Sir John. The Serpent Power., New Delhi: New Age Books; 2012.
37. Erbl-Roth G. Uberden Reissnerchen Fadender Wirbeltiere. Zeitscheiff Fur Mickr. Anat. Forsh. 1951;57:180–95.
38. Hess J. Studies concerning the function of the complex subcommissural organ-liquor fibre: The binding of the liquor fibre to pyrocatechin derivatives and its functional aspects.Brain Research. 1973;58:303–312.
39. Bakhtinov AP. Ultrastructure of cells of the human intraspinal organ Arkh. Anat. Gistol. Embriol. 1984;87:26-32.
40. Studnička FK. Der Reissnersche Fadenim Zentralkanal des Rückenmarks und sein Verhalten in dem Ventriculus terminalis. Sitz Ber Kgl BöhmGesellsch Wiss Prag. 1899;36:1–10.
41. De Vernejoul P et al. Study of acupuncture meridians using radioactive tracers. Bulletin de L'Academie Nationale de Medicine. 1985;169:1071–75.
42. Soh KS. Bonghan duct and acupuncture meridian as optical channel of biophotons. Journal of the Korean Physical Society. 2004;45:1196–1198.
43. Lee et al. Novel anatomic structures in the brain and spinal cord of rabbit that may belong to the Bonghan system of potential acupuncture meridians. J. Acupunct. Meridian Stud; 2008.
DOI: 10.1016/S2005-2901(09)60004-2.
44. Ponce C. Kabbalah. Wheaton, Ill.: The Theosophical Publishing House; 1978.
45. Wile L. Near-death experiences: A speculative neural model." Journal of Near-Death Studies. 1994;12:133–42.
46. Miller A. 137: Jung, Pauli, and the Pursuit of a Scientific Obsession W. W. Norton & Company; 2010.
47. Schrödinger E. What is Life? and Mind and Matter. Cambridge: Cambridge University; 1969.
48. Marin JM Mysticism' in quantum mechanics: The forgotten controversy. European Journal of Physics; 2009.
DOI: 10.1088/0143-0807/30/4/014
49. Plotnitsky A. Mysteries without Mysticism and Correlations without Correlata: On Quantum Knowledge and Knowledge in General; 2003.
DOI.org/10.1023/A:102606521
50. Engel G, et al. Evidence for wavelike energy transfer through quantum coherence in photosynthetic systems. Nature. 2007;446:782–786.

51. Tscherbul T. Quantum coherence effects in natural light-induced processes: cis–trans photoisomerization of model retinal under incoherent excitation. Physical Chemistry Chemical Physics; 2015.
DOI:10.1039/5CP01388G
52. Hamish G, et al. The quantum needle of the avian magnetic compass. PNAS; 2016.
DOI.ORG/10.1073/PNAS.1600341113
53. Lambert N, et al. Quantum Biology. Nature Physics. 2013;9:10–18.
54. Mohseni M, et al. (eds.) Quantum Effects in Biology. Cambridge: Cambridge University Press; 2014.
55. Stojkovic S. Vanishing dimensions: theory and phenomenology. 2013;arXiv:1304.6444
56. Akle V. F-Spondin/spn1b Expression Patterns in Developing and Adult Zebrafish. PLOS; 2012.
DOI.ORG/10.1371/JOURNAL.PONE.0037593
57. Carrillo GL. F-spondin Is Essential for Maintaining Circadian Rhythms. Front Neural Circuits. 2018;12:13.
DOI: 10.3389/fncir.2018.00013.
58. Hafez DM, et al. F-spondin gene transfer improves memory performance and reduces amyloid-β levels in mice. Neuroscience. 2012;223:465–472.

Biography of author(s)



L. Wile
Chaikin-Wile Foundation, 8 Winged Foot Blvd., Bloomfield CT, 06002, USA.

He received a B.S. in physics from Union College (1971), an M.D. from the University of Connecticut School of Medicine (1976), a post-doctoral fellowship from Yale University School of Medicine (1979) and an M.A. in philosophy from the University of Massachusetts (1991). He is a Diplomate of the American Board of Psychiatry and Neurology. He is currently serving as the President of the Chaikin-Wile Foundation and is leading a multidisciplinary team at Boston University and the University of Connecticut dedicated to the exploration of Reissner's fiber. He is the author of the Jaynes Legacy: Shining New Light Through the Cracks of the Bicameral Mind.

© Copyright (2021): Author(s). The licensee is the publisher (Book Publisher International).

DISCLAIMER
This chapter is an extended version of the article published by the same author(s) in the following journal.
Annal Behav Neurosci, 1(1):56-65, 2018.

Chapter 6
Print ISBN: 978-93-90516-93-3, eBook ISBN: 978-93-90516-94-0

Emphasizing on Reissner's Fiber: A Forgotten Pathway for Exploring Consciousness

Lawrence Wile[1*]

DOI: 10.9734/bpi/rrab/v5/7360D

ABSTRACT

One of the most remarkable ongoing stories in the history of neuroscience is the nearly universal neglect of what is arguably the most strategically located structure in the central nervous system (CNS), an enigmatic, thread-like aggregation of glycoproteins originating from the center of the brain called Reissner's fiber (RF). It is an anomaly, which, because it lies beyond the grasp of conventional theoretical and technological tools, has been rendered virtually invisible to neuroscientists. New tools, however, are poised to reveal the secrets of this unique, highly conserved structure which runs through the entire length of the fluid-filled cavities and passageways of the CNS. This paper proposes that an enigmatic, thread-like aggregation of glycoproteins originating from the center of the brain called Reissner's fiber (RF), explored with quantum theoretical tools and seen through the lens of its possible recent evolutionary degeneration, can provide a new intellectual and physical pathway toward a deeper understanding of consciousness.

Keywords: *Consciousness; quantum neurobiology; yoga.*

1. INTRODUCTION

Christof Koch, reflecting mainstream scientific opinion, recently compared quantum mechanical theories of consciousness to the "sloppy, nonsensical theories" that Wolfgang Pauli would disdainfully dismiss with the quip, "It's not even wrong." Ironically, apparently unbeknownst to Koch, Pauli himself believed that quantum mechanics points the way toward a unity of mind and matter, and a synthesis of the mystical and the scientific [1,2,3,4].

"An even more far-fetched idea," Koch continued, "holds that consciousness emerged only a few thousand years ago, when humans realized that the voices in their head came not from the gods but from their own internal spoken narrative [5]. “While the idea as stated by Koch does indeed appear implausible, it is based on two fundamental misunderstandings of the theory proposed by Julian Jaynes in *The Origin of Consciousness in the Breakdown of the Bicameral Mind.* First, owing to ambiguities of the definition of consciousness, Koch misunderstood Jaynes' theory to be an explanation of the adaptive reactivity displayed by primitive organisms such as worms. However, the consciousness that Jaynes sought to explain was the "introspectable mind-space" within which humans can exercise the powers of imagination, reason and intuition. This qualitatively new, self-aware, creative consciousness, Jaynes emphasized, is distinct from the cognitive, emotional, and sensory capacities that we share with other animals. Consciousness as a subject of investigation has indeed had something of a torrid time within the interdisciplinary field of cognitive science [6].

Koch's disdain for the notion that consciousness originated several thousand years ago derives from his belief that, "given the lack of a clear and compelling Rubicon separating simple from complex animals and simple from complex behaviors, the belief that only humans are capable of experiencing anything consciously seems preposterous" [7]. However, the proposed evolutionary leap taken by humans across the Rubicon did not occur at the boundary between simple and the complex

[1]*Chaikin-Wile Foundation, 8 Winged Foot Blvd., Bloomfield CT, 06002, USA.*
**Corresponding author: E-mail: chaikinwile@aol.com;*

behaviors. While it is true that every species is capable of feats that would be attributed to consciousness if performed by humans, it is also true that only human consciousness has transcended its undeniable animal origins and launched on a unique, ever accelerating trajectory several thousand years ago based on abstract thinking. It has been pointed out, however, that self knowledge does not easily explain consciousness [8,9]. While other animals have repeated their complex programmed behaviors for hundreds of millions of years, humans have taken creative leaps from fire to atomic energy, from camels to space shuttles, and from ink and quills to terabyte home computers. We now stand at the threshold of genetically engineering our own biological evolution and nanoengineering our neural circuits. Like our bicameral ancestors, we are poised for unimaginable leaps.

Second, it was not our ancestors' realization that the voices in their heads came not from the gods but from their own internal spoken narrative that generated our higher powers of consciousness. The capacity for realizations itself--the ability to imagine the nature of gods and the nature of internal narratives, and the ability to reason and decide which alternative is true--is the defining property of human consciousness not its cause. The precipitating factor that led to the origin of human consciousness, according to Jaynes, was the breakdown of the "bicameral mind" which fed auditory messages from the right side of the brain fed to the left side via the anterior commissure. The neural signals associated with this "bicameral mind" were transmitted automatically to the motor pathways. Our bicameral ancestors, therefore, lacked the capacity to contemplate and chose alternative thoughts and behaviors. They heard voices and blindly obeyed. Complex behaviors performed by our bicameral ancestors were programmed like those of ants and bees.

Rather than dismiss ideas about quantum theories of consciousness, or about the possible recent evolutionary transformation of human consciousness as "intellectual flapdoodle," let us be guided by experimental results. This paper proposes that an enigmatic, thread-like aggregation of glycoproteins originating from the center of the brain called Reissner's fiber (RF), explored with quantum theoretical tools and seen through the lens of its possible recent evolutionary degeneration, can provide a new intellectual and physical pathway toward a deeper understanding of consciousness.

2. REISSNER'S FIBER: THE DEVIL ACCORDING TO CHARLES BAUDELAIRE?

Reflecting on science's neglect of RF, two historians of neuroscience, Regis Olry and Duane Haines, published an article titled, "Reissner's Fibre: The Exception Which Proves the Rule, or the Devil According to Charles Baudelaire" [10] After reviewing the roots and evolution of various terms used by neuroscientists, they concluded that RF is a counterexample to the "never-ending updating of terms that had been coined by various researchers." A line from the nineteenth-century poet's story "The Generous Gambler" inspired their metaphorical identification of RF with the Devil: "My dear brethren, do not ever forget, when you hear the progress of lights praised, that *the loveliest trick of the Devil is to persuade you that he doesn't exist.*" The preacher who speaks these words and who is "more subtle that the rest of the human herd" tells his congregation that the Devil attends all scientific meetings to shape the participant's thoughts and opinions while professing that his supreme goal is to bring about the "destruction of Superstition."

Olry and Haines' playful metaphor is apt. For sixty years after its discovery in 1860, neuroscientists debated whether RF exists. We still do not know if it exists beyond fetal stages in humans. Our knowledge of this highly conserved, strategically located structure has improved very little.

Reissner's fiber has been able to play the trick of convincing neuroscientists that it doesn't exist because of its physical elusiveness, inconclusive evidence of its persistence past late fetal development, and its virtual invisibility when seen through the lens of the current neuroscientific paradigm. For thousands of years, fishermen had cut off the heads of their catch of the day, but it was not until 1860 that Ernst Reissner observed a translucent thread finer than a human hair in the central canal of a lamprey [11]. In 1863, Karl Kutschin confirmed Reissner's discovery, naming it "Reissner's fiber" [12] Although Reissner had explicitly distinguished the fiber's "constant features" with "the irregular masses which occasionally fill the central canal and have been mentioned by several investigators to occur in the spinal cord in other animals or in man," subsequent investigators,

beginning with Steida in 1868, asserted that the fiber is an artifact [13]. Anatomists at the time.—Viault in 1876 [14], Rohon in 1877 [15], Sanders in 1878 and 1894 [16,17], and Gadow [18] in 1891—were quick to embrace Steida's skepticism.

In the spring of 1899, Porter Sargent, a twenty-seven year old doctoral candidate at Harvard sat examining lengthwise sections of the spinal cord of a lamprey. Curious about the thread-like structure he saw within the central canal, he searched the scientific literature and was astounded that, "so peculiar and conspicuous a structure as Reissner's fiber, which is of so great importance in the nervous anatomy as to persist throughout the vertebrate series, should've remained so little known for forty years after its discovery." Similarly, he was dismayed that the ventricles, their lining and content, had been almost entirely dismissed. Sargent set out to disprove the consensus that RF was an artifact. For six years, Sargent devoted himself to investigating the fiber in nearly one hundred species of fish, focusing on species in early stages of evolutionary and embryological development. He laid the foundation for the modern anatomical understanding of RF as a biological structure that runs from the SCO through the third and fourth cerebral ventricles and central canal and terminates in the terminal ventricle.

Based on his conclusion that RF links the visual and motor pathways, Sargent proposed that it is a "short circuit" for transmitting "optical motor reflexes" which conferred an advantage in the Darwinian struggle for survival. He performed experiments in which he severed RF in small sharks--whose fibers range between 10-20 microns in diameter--which, he claimed, supported his hypothesis about the fiber's function. His observations of poorly developed RFs in kittens and mouse pups, who are born blind also supported his hypothesis. Curiously, Kalberlah, [19] and Streeter in 1903 [20], continued to agree with previous erroneous characterizations of the fiber as cellular debris or coagulated proteins in the cerebrospinal fluid (CSF).

Sargent concluded his 1905 paper by saying, "The conclusions and the discussion of the results and bearings of this research are reserved for the second part of this paper dealing with the higher vertebrates. This is already well advanced, and it is hoped will appear in about a year" [21]. However, following a contentious defense of his doctoral dissertation, Sargent, decided that path of freedom and discovery lay outside the walls of Harvard. He went on to become an iconoclastic social critic, poet and educator. His final publication in 1954 was "Is Poetry a Secretion?"

For several years following his hasty, bitter departure from the scientific community, Sargent's hypothesis that RF is a novel pathway for high-speed transmission of nerve signals was generally accepted [22]. In 1908, inspired by Sargent's research and giving "greatest deference" to his opinions regarding the fiber's function, pioneering neurosurgeon Sir Victor Horsley undertook an investigation of RF in apes [23]. Owing to the fragility of the fiber and its elastic recoil, Horsley was able to examine it adequately in sagittal sections of the spinal cord in only one specimen of *Maccacus cynomolgus.* He showed that the fiber lacked the structure of nerves and did not display Wallerian degeneration. He urged reinvestigation of the fiber before dismissing it as vestigial. That same year, Ludwig Edinger, one of the founders of modern neuroanatomy, disputed RF's existence as an actual biological structure [24].

RF's transition from frequent target of scientific investigations to oblivion was precipitated by two significant developments. First, signals in the brain were coming to be understood as electrical spikes generated by ion flows across polarized axonal membranes. As the fuzzy boundary separating axons from RF became distinct, the fiber ceased to be considered as a physical correlate of consciousness. Second, investigations of human specimens suggest that RF's activity peaks during the fifth month of human fetal development and completely degenerates by birth. Similarly, investigation of the SCO suggested that it regresses during late fetal development and becomes a vestigial islet by late infancy. A few "black swans" --the observation of the fiber was in a 14-year-old teenager in 1922 by Eric Agdhur [25], the ambiguous observation of the fiber in an infant by Lucas Keene in 1935 [26] and the observation of a fully developed SCO in a 60-year-old man by Bosque Gomez in 1961 [27] --have not altered the textbook consensus that Reissner's fiber does not exist in human adults.

RF could have thus far evaded observation in human adults because of its rapid post-mortem degeneration and inaccessibility. During life, CSF flow produced by cardiac and respiratory rhythms ceases. The rhythmic beating of cilia along the ventricular surface, which keeps the fiber afloat, also stops. Shortly after death, fiber sinks to the surface of the neurocele where enzymes released by dying cells break it down. Unless the deceased's head were ghoulishly frozen at the moment of death, the fiber could play its devilish trick.

Observation of the fiber in living subjects is also problematic. The third and fourth ventricles are generally accessible to neurosurgeons only via relatively low-resolution fiber optic devices. Current neuroimaging devices such MRI and PET similarly lack sufficient resolution to detect the fiber. The words of the French investigator of RF, Etienne-Jules Legait, from 1942, remain true today: "when its existence is denied, this fact should be carefully accepted and discussed: one could not take it into account if fixation is uncertain" [28].

In 1909, George Nicholls seized on Sargent's mistaken characterization of RF as an axon and asserted that his hypothesis that RF transmits signals was therefore wrong. Nicholls also mistakenly claimed that, contrary to Reissner's statements that the fiber he had observed displays a stronger refractive capacity than axis-cylinders and always displays constant features in contrast to the variations of form characteristic of axis-cylinders, he had believed the fiber was simply a nerve fiber.

Ironically, Nicholls' ostensible refutation of Sargent's hypothesis was based on his own mischaracterization of the fiber as a "coalescence of cilia-like processes springing from cells," mostly from the SCO, but from other ependymal cells lining the central canal as well. He went on to describe the fiber as a "thread of protoplasm" whose mode of contraction is "paralleled only in the scarcely modified protoplasm which forms the stalk of certain Protozoa." He proposed that the fiber is a strand of connective tissue which transmits changes in its tension to sensors in the SCO to regulate the flexure of fish [29,30]. As 20th century neuroscientists focused on action potentials in axons and interpreted the absence of evidence of RF in adult humans as evidence of its absence, the fiber drifted to the backwaters, where it remained for fifty years. However, while RF attracted little interest during the second half of the 20th century, a few interesting findings emerged. In 1951, G. Erbl-Roth observed that fiber is hollow in fresh specimens. She proposed that the fiber serves as a transporting duct [31].

Under an electron microscope, the 2-micron diameter fiber appears as parallel 5-10 nanometer filaments embedded in a complex matrix. Spaces within this matrix material contain tiny spheres surrounded by a tri-layered membrane filled with a homogenous, granular substance, [32]. Extending from RF to the surface of the neurocele are numerous filaments [33,34,35,36,37,38]. The fiber's rate of growth correlates with photoperiods and the activity of the pineal gland as measured autoradiographically following the injection of the radioactive isotope S^{35}, which were incorporated into the cysteine-rich fiber [39,40,41].

The function of RF is unclear. The SCO-RF complex regulates fluid and electrolyte balance [42,43,44,45]. It is involved in neurogenesis and neural development [46,47,48]. This function is mediated, in part, by extracellular matrix (ECM) proteins such as SCO-spondin [49] and f-spondin [50], potent influencers of axonal guidance and neurite outgrowth. RF binds neurotransmitters including adrenaline, noradrenalin and serotonin. However, without a biophysical model that could integrate this interaction with possible physical correlates of consciousness, researchers have concluded that the fiber is merely a waste disposal system that detoxifies the CSF [51,52,53,54]. RF has also been implicated in the pathogenesis of hydrocephalus [55,56,57,58,59,60].

3. A CIRCUMNEUROCELIC SENSORY SYSTEM

In 1913, Tretjakov proposed that the cilia and CSF-contacting neurons surrounding RF form a “central sense organ” [61]. In 1921, William Kolmer elaborated on Tretjakov's proposal, comparing RF to the gel-like tectorial membrane of the inner ear which lies beneath Reissner’s membrane. He named this novel sensory system the "sagittal organ" [62].

The following year, Agdhur, who, together with Kolmer, discovered specialized sensory neurons bordering the central canal, (Kolmer-Agdhur cells), proposed a similar "ependymal sensory organ." He published a 137-page manuscript dealing with this "central sensory organ in vertebrates." However, contrary to Kolmer, Agdhur concluded that RF is not part of this system [63]. He therefore refused to accept the term *saggital organ* and suggested that the synonymous term *median organ* be used instead. Agdhur performed extensive experiments involving the severing of RF in fish. While acknowledging that his results were difficult to interpret because his technique resulted in the severing of major pathways in the spinal cord, he rejected Sargent's, and Dendy and Nicholls' theories.

Nine years after Agdhur's criticisms, Kolmer published the results of his research on the structure he had named the *saggital organ*. Based on his extensive observations of lizards and snakes--as well as a detailed study of rhesus monkeys--Kolmer rejected Agdhur's criticisms. Observing RF in the regenerating tails of lizards and noting the enormous length of the fiber in giant snakes, Kolmer concluded that the coordinated beating of the cilia propels the ever growing fiber along the central canal of the spinal cord. He continued to believe that RF transmits vibrations to inner-directed sensory system. However, as RF fell through the conceptual and technological nets of 20th century science, so did the hypotheses of Tretjakov and Kolmer.

Nevertheless, subsequent research has led to a reassessment. The surface of the neurocele is lined with cilia and cerebrospinal CSF-contacting neurons that share a common embryological origin and anatomical features with the external sense organs [64,65]. They contain photoreceptor molecules homologous to pineal and retinal opsins [66,67,68]. A thin layer of 5-nanometer, striated filaments, similar to ependymally derived cells of the retina are located on the apical surface of the central canal [69]. CSF- contacting neurons include those which project from the dorsal raphe nucleus into the third ventricle, a site of action of LSD [70] and the key neural input to the SCO [71], and opiate receptors surrounding the periaqueductal gray area [72]. In addition to cilia and CSF-contacting neurons are various circumventricular organs. These structures, including the SCO, organum vasculosum of the lamina terminalis and the pineal gland, lack the normal blood brain barrier, and have a variety of sensory and secretory functions [73]. While it is generally assumed that our external sense organs are the only source of percepts, it possible that RF and a circumneurocelic sensory system (CNSS) surrounding it could generate percepts as well. Such a system would supplement the interoceptive system which provides cortical images of afferents arising from the autonomic nervous system [74].

4. A NEW CONCEPTUAL FRAMEWORK

While RF is currently disconnected from the prevailing neuroscientific paradigm that understands consciousness as an emergent property of the integrated firings of neurons, two, possibly dovetailing, speculative models might incorporate it. First, because the fiber consists of 2-5 nanometer filaments, it is an interesting site for exploring possible quantum effects. It is directly visible, floating in the clear CSF, thereby accessible to measurements. While current theories of decoherence predict that RF's quantum properties will dissipate nearly instantaneously in the warm wet brain through a process whereby information becomes entangled with its environment and thereby lost, there is some evidence to suggest that biological systems can amplify and control quantum effects [75]. RF is located within a cavity surrounded by afferent and efferent systems with quantum mechanical sensitivity that is analogous to quantum cavity electrodynamical systems of feedback and control that are being used to amplify quantum effects [76]. Information detected by the inner sensory system surrounding the fiber could be fed back to it to achieve recoherence. Neuroimaging devices are on the threshold of measuring quantum coherences and entanglements in the brain and offer the possibility of consciously enhancing them.

Second, it is possible that descriptions of a spiritual energy running through the center of the spine by our ancient ancestors were based on interoceptions of RF by a CNSS. In 1939, Theos Bernard, the first American initiated into Tantric yoga practices by the highest lama in Tibet, wrote the following in his memoir about his travels to India and Tibet titled *Heaven Lies Within Us*: "Inside this central (*Sushumna*) nadi, the Yogi identifies an invisible nadi known in the West as the fibre of Reissner, but which is known here as *Chittra* (the Heavenly Passage, in Sanskrit)" [77]. The origin of mystical

traditions might be accounts of the witnessing of the evolutionary degeneration of RF that is now typically mirrored by its fetal regression [78].

According to mystical lore, descriptions of the "subtle anatomy" originated over 6,000 years ago. This origination is 3,000 years earlier than Jaynes' hypothesized breakdown of the bicameral mind, but its neural basis is similar. RF, which preferentially attaches to the right side of the SCO [79], could transmit perceptions, including visual images of the RF apparatus and possibly visual signs and symbols corresponding to auditory elements of the bicameral mind, from the right side of the brain to the left side across the posterior commissure. Another interesting feature of the SCO-RF complex that lends support to RF's hypothesized role in the origin of mystical traditions is that the SCO marks the embryological boundary between the motor-sensory area (chordencephalon), and analyzer-correlator areas of the brain (acrencephalon) [80]. RF could have provided novel neural inputs into the process of abstract thinking that Einstein once described as creatively reproducing and recombining visual and motor images [81].

New techniques for epigenetic research could reveal the epigenome of our ancient ancestors from anthropoid apes to Neanderthals to modern man and test the hypothesis that RF underwent an evolutionarily recent epigenetic degeneration [82]. By employing newly developed techniques to regulate the expression of the genes responsible for the formation of RF we could regenerate it.

Individuals who might have partial, transient, or pathological manifestations of RF could also help us explore the fiber's role in mystical experiences. Such individuals include those who have reported awakenings of *Qi* or *kundalini*, including its pathological manifestations in Qi-Gong and kundalini syndromes [83], and schizophrenia [84]. Individuals who describe awakenings of *Qi* and *kundalini* as a stream of light or electricity traveling inside their spines could similarly be describing the activity of RF. Schizophrenics whose prodromal phase often overlaps mystical experiences might reflect late degeneration of RF. Anti-SCO antibodies correlate with exacerbations of schizophrenia [85].

In 1960, a North Korean scientist named Kim Bonghan began investigating the possible neuroanatomical basis of acupuncture [86]. After injecting radioactive phosphorous (P^{32}) into acupuncture points on a rabbit’s abdomen corresponding to the Governing Vessel, he traced its flow using autoradiography. He found that injections into acupuncture points followed meridians along a novel network of ducts. Injections into other sites dispersed. A photomicrograph of Bonghan's results shows a hollow, thread-like structure inside the central canal. Apparently unaware of RF he named it after himself, the neural Bonghan duct.

Bonghan was praised by the North Korean government for discovering a “monumental theory in global science,” and was elevated to a high position in the government. However, shortly thereafter, amidst allegations that he'd withheld details of his methodology, which prevented other scientists from replicating his results, he was discredited and banished. The scientific community dismissed Bonghan's findings for several decades. Then at the turn of the twentieth century, neuroscientists at the Seoul National University using new anatomical techniques including fluorescent magnetic nanoparticles and confocal laser scanning microscopy "rediscovered" the network of Bonghan ducts. According to Kwang-Sup Soh at the Biomedical Physics Laboratory, these microscopic ducts act as optical channels for coherent biophotons. In 2008, his team reported the discovery of a "novel thread-like structure" in the cerebral ventricles and central canal in a rabbit [87]. Apparently unaware of the "neural Bonghan duct" or RF, they believed that they had discovered a "novel thread-like structure."

5. CONVERGENCE OF SCIENCE AND MYSTICISM

Parallels between mysticism and modern physics have been contemplated since the inception of quantum mechanics. Pauli believed that “a synthesis embracing both rational understanding and the mystical experience of unity, to be the mythos, spoken or unspoken, of our present day.” Such a synthesis would be achieved when "natural sciences will out of themselves bring forth a counter pole in their adherents, which connects to the old mystic elements." Erwin Schrodinger proposed a careful "blood transfusion of Eastern thought." But he warned, "blood transfusion always needs great

precaution to prevent clotting. We do not wish to lose the logical precision that our scientific thought has reached, and that is unparalleled anywhere at any epoch."

A fundamental difference between physics and mysticism is that physicists know deeper realities as mathematical abstractions while mystics claim to experience them directly. According to quantum orthodoxy, there is no quantum ontology. Reality exists as abstractions, "clouds of probability," before an observation. "There is no quantum world. There is only an abstract quantum description," said Niels Bohr. According to Werner Heisenberg, we couldn't know the quantum world even if it was real: "The use of classical concepts is finally a consequence of the general way of thinking. There is no use in discussing what could be done if we were other beings than we are." However, it is possible that ancient mystics were beings other that we are. They were witnesses of the evolutionary degeneration of RF. By regenerating RF, and measuring and amplifying its quantum effects, we can create a counter pole out of the natural sciences and connect them to old mystic elements.

For the past 15 years, my team of scientists, led by Professor Irina Zhdanova, Department of Anatomy and Neurobiology, Boston University School of Medicine and Professor Alexander Sergienko, Department of Electrical and Computer Engineering and Department of Physics, Boston University Photonics Center, has been developing an interdisciplinary study of RF. First, our team developed a transgenic zebrafish in which the expression of green fluorescent protein (GFP) is under control of the promoter for F-spondin, an extracellular matrix protein associated with RF that is involved in neurogenesis, neuronal guidance and neuroplasticity. This effective visual tool has allowed for detailed neuroanatomical localization of F-spondin, and helps us focus on RF in living animals. We can further explore RF's role in neurogenesis [88] and the pathogenesis of Alzheimer's disease [89,90] and its links to the ECM. It also allows us to investigate the epigenetic factors involved in the regulation of the f-spondin gene.

Next, we developed a novel microspectrometer to analyze biophotonic emissions from RF in living zebrafish larvae and similarly transparent adult zebrafish mutants (Caspar line) using the technology of correlated photon counting with superconducting single-photon detectors coupled to a time-correlated counting system with picosecond resolution. Our apparatus is equipped with femtosecond pulsed infrared lasers to perform photon echo-based experiments similar to those recently used to reveal the quantum mechanisms involved in light harvesting during photosynthesis. Once we achieve our goal of recording photonic signals from RF, we will be able to explore micro-changes in its conformation, relaxation times of internal excitation, fluctuations of the intensity and direction of photon emissions, and possible quantum coherences and entanglements. Using transgenic zebrafish with genetically encoded calcium indicators, we can document neuronal activity associated with quantum behaviors in the fiber. Our zebrafish facility is also equipped for video monitoring and computerized analysis of behaviors.

In 1958, Pauli presented a new theory of particle physics. Following his hour-long presentation, he remarked that his theory might appear "somewhat crazy." Bohr replied, "We are all agreed that your theory is crazy, Pauli. The question which divides us is whether it is crazy enough to have a chance of being correct. My own feeling is that it is not crazy enough." The hypothesis that RF could provide the physical basis of a quantum biophysical synthesis of science and mysticism--as envisioned by Pauli--lacks that shortcoming. It is also, for the first time in history, becoming scientifically testable.

Cognizant of the wisdom of the old adage, "The Devil is in details," our team is acquiring data about RF's subtlest activities and developing mathematical models for understanding macroscopic quantum effects. Whether these details will reveal merely extraneous noise of a waste disposal and transport system, the key to new treatments for degenerative and traumatic brain disorders and hydrocephalous, anomalies that seed a new quantum neurobiological paradigm or the rediscovery of the lost subtle anatomy remains to be seen.

6. CONCLUSION

The confluence of the mysteries surrounding mystical traditions, quantum mechanics and consciousness has contributed little to the advancement of science. However, the identification of RF

with the central axis of the “subtle body,” the connection between the human microcosm and the supernal macrocosm, described by Indian, Chinese and other mystical traditions, might contribute to our understanding of the evolution of consciousness and the ontology of quanta. If mystical traditions are true, the restoration of the function of RF and its enhancement based on a neurophenomenology using new generations of neuroimaging devices holds the promise of freedom, immortality and cosmic redemption.

COMPETING INTERESTS

Author has declared that no competing interests exist.

REFERENCES

1. Atmanspacher H, Primas H. The Hidden Side of Wolfgang Pauli: An Eminent Physicist's Encounter with Depth Psychology, Journal of Consciousness Studies. 1996;3(12):112-26.
2. Atmanspacher H. Pauli's Ideas on Mind and Matter in the Context of Contemporary Science. Journal of Consciousness Studies. 2000;13(31):5-50.
3. Gieser S. The Innermost Kernel. Berlin: Springer; 2005.
4. Marin JM. Mysticism in quantum mechanics: the forgotten controversy, European Journal of Physics. 2009;30:807-22.
5. Koch C. Consciousness Redux. Scientific American Mind. 2014;25(3):26-29.
6. Pitman MM. Consciousness studies: Research prospects in the' cradle of human consciousness'. Alternation. 2003;10(1):271-291.
7. Koch C. Consciousness Redux. Scientific American Mind. 2013;25(1):27-31.
8. Crick F, Koch C. Toward a neurobiological theory of consciousness. Seminars in the Neurosciences. 1990;2:263–275.
9. Graziano MS, Kastner S. Human consciousness and its relationship to social neuroscience: a novel hypothesis. Cognitive neuroscience. 2011;2(2):98-113.
10. Olry R, Haines D. Reissner's Fiber: The exception which proves the rule, or the Devil according to Charles Baudelaire?, Journal of the History of the Neurosciences. 2003;12(1):73-75.
11. Reissner E. Beitrage zur Kenntiss vom Bau des Ruskenmarks von Petromyzon fluvatis, Arch. Anat. Physiol. 1860;445-488.
12. Kutschin W. Uber den Bau Ruckenmark von Neunauges. Abstract by Steida, Archiv mikr. Anat.. Bd. 1863;2.
13. Steida L. Studien uber das centrale Nervensystem der Wirbelthiere. Zeits f. wiss. Zool, Bd. 1868;19.
14. Viault F. Recherches histologiques sur la Strucuture des Centres nerveux des Plagiostomes, Arch. Zool. exper. gen, Tom. 1876;5.
15. Rohon JV. Das Centralorgan des Nervensystems der Selachier, Deks Acad. Wiss. Wien Math. Naturw, Bd. 1870;38.
16. Sanders A. Contribution to the anatomy of the central nervous system in vertebrate animals, Philosophical Transactions Royal Society London. 1878;189.
17. Sanders A. Researches in the nervous system of Myxine glutinosa, Philsophical Transactions Royal Society London; 1894.
18. Gadow, H.Bronn’s Klaussen und Ordnungen des Thierrechs, Bd.6 1891;
19. Kalberlah A. Uber das Ruckenmark der Plagiostomes. Ein Beitrag zur vergleichenden Anatomie des Centralnervensystems, Zeitschr. f. ges. Naturewiss, Bd. 1900;73.
20. Streeter GL. The structure of the spinal cord of the ostrich, American Journal of Anatomy. 1902; 3.
21. Sargent PE. The optic reflex apparatus of vertebrates for short-circuit transmission of motor reflexes through Reissner's fiber: Its morphology, ontogeny, phylogeny and function. Part I. The fish-like vertebrates, Bulletin of the Museum of Comparative Zoology at Harvard University. 1905;45:1-256.
22. Sherrington C. Integrative Action of the Nervous System, New Haven: Yale University Press; 1920.
23. Horsley V. Note on the existence of Reissner's fiber in higher vertebrates, Brain. 1908;31(1): 147-59.

24. Edinger L. Vorlesungen uber den Bau der nervosen Centralorgane des Menschen und der Tiere, Vergl. Anat. des Gehirnes, Bd 2, 7 Aufl. Leipzig; 1908.
25. Agdhur E. Uber ein zentrales Sinnesorgan (?) bei den Vertebraten. Zischr. Anat. Entwickelungesch. 1922;66:223-30.
26. Keene MLF, Hewer EE. The subcommissural organ and the mesocoelic recess in the human brain, together with a note on Reissner's fibre Journal of anatomy, (London). 1935;69:501-7.
27. Gomez B, Benito-Aaranz E, Rodriguez M. The subcommissural organ in the grown-up man Acta. Anat. 1961;44:98-103.
28. Legait G. Les Organes epenymaires du troisemeventricle. L'organesou-commissural. Lorgane sub-fornical. L'organe para-ventriculare. Nancy, Georges Thomas. 1942;72-104.
29. Nicholls G. The function of Reissner's fiber and the ependymal groove, Nature. 1909;82.
30. Nicholls G. Some experiments on the nature and function of Reissner's fiber, Journal of Comparative Neurology. 1917;27(2):119-199.
31. Erbl-Roth G. Uber den Reissnerchen Faden derWirbeltiere. Zeitscheiff Fur Mickr. Anat. Forsh. 1951;57:180–95.
32. Kohno K. Electron microscope study of Reissner's fiber, Z. Zellforsch. 1969;94:565-573.
33. Jordan, H. Concerning Reissner's fiber in teleosts, Journal of Comparative Neurology, 1917;216-277
34. Jordan, H. The structure and staingin of the Reissner's fiber apparatus particularly the subcommissural organ, Jounral of Anatomy, 1925; 427-443.
35. Schinko I, Weindl A. Scanning electron microscopy of Reissner's fiber. Nova Acta. Leopol. Leipzig Suppl. 1977;9:169–72.
36. Kumar P, Kumar S. Ultrastructural studies on the ependymal secretion and formation of Reissner's fiber in the goat, Indian Journal of Animal Sciences. 1998;68(7):597–600.
37. Woolam DHM, Collins P. Reissner's fibre in the rat: A scanning and transmission electron microscope study, Journal Anatomy London. 1980;131:135-43.
38. Castenholz A, Zoltzer H. Formation and morphology of Reissner's fiber in primates. A scanning electron microscopic study, Cell and Tissue Research. 1980;207:43–53.
39. Diederen JB. Influence of light and darkness on secretory activity of the subcommissural organ and on the growth rat of Reissner's fiber, Z. Zellfrorsch. U. Mikrosk. Anat. 1973;139:83-94.
40. Sterba G. Incorporation of S^{35} into the subcommissural organ and Reissner's fiber Nature. 1967;216:504-13.
41. Wakahara L. Daily variation in mitotic rate in tail-fin epidermis of larval Xenopus laevis and its modification by pineal organ, subcommissural organ system and photoperiods. Neuroendocrinology. 1972;121:267–77.
42. Farrell G. The physiological factors which influence the secretion of aldosterone, Rec. Progr. Hormone Res. 1959;15:275–310.
43. Gilbert GJ. The subcommissural organ: A regulator of thirst, American Journal of Physiology. 1956;191:243–47.
44. Gilbert GJ. The subcommissural organ and water-electrolyte metabolism, in Thirst. Proc. 1st Symposium on thirst regulation of body water (Oxford: Pergamon Press; 1964.
45. Palkovits M. Morphology and function of the subcommissural organ, Studia Biologica Hungarica. 1965;4:1–105.
46. El-Bitar F, Dastugue, Meiniel A. Neuroblastoma B104 cell line as a model for analysis of neurite outgrowth and neuronal aggregation induced by Reissner's fiber material, Cell and Tissue Research. 1999;298(2):233–42.
47. Monnerie H, et al. Reissner's fiber promotes neural aggregation and influences neuritic outgrowth in vitro, Cell and Tissue Research. 1997;287(2):285–95.
48. Gobron S, et al. SCO-spondin: A new member of the thrombospondin family secreted by the subcommissural organ is a candidate in the modulation of neuronal aggregation, Journal of Cell Science. 1996;109(5):1053–61.
49. Burstyn-Cohen T, Frumkin A. Accumulation of F-spondin in injured peripheral nerve promotes the outgrowth of sensory axons, Journal of Neuroscience. 1998;18(21):8875–8885.
50. Caprile T, Hein S. Reissner's fiber binds and transports away monoamines present in the cerebrospinal fluid, Brain Res Mol Brain Res. 2003;110(2):177-92.

51. Ermisch A, Sterba G, Hess J. Untersuchungen zur Klarung der Funktion des Reissnerschen Fadens. In vitro Bindung von Noradrenalin, Adrenalin und Serotonin, Experientia. 1970;26: 1319–21.
52. Hess J, Sterba G. Die Bindungsfahigkeit des Reissnerschen Fadens fur Adrenalin und Noradrenalin in vivo, Acta. Biol. Med. Germ. 1972;28:849–51.
53. Hess J. Studies concerning the function of the complex subcommissural organ-liquor fibre: The binding of the liquor fibre to pyrocatechin derivatives and its functional aspects, Brain Research. 1973;58:303-12.
54. Rodriguez S, Caprile T. Functional aspects of the subcommissural organ-Reissner's fiber complex with emphasis in the clearance of brain monoamines. Microsc Res Tech. 2001;52(5): 564-72.
55. Castaneyra-Perdomo A, Meyer G. Alterations of the subcommissural organ in the hydrocephalic human fetal brain, Dev. Brain Res. 1994;79:316–20.
56. Fernández-Llebrez P, et al. MSx-1-deficient mice fail to form prosomere derivatives, subcommissural organ and develop hydrocephalus, J Neuropathol Exp Neurol. 2004;63(6):574-86.
57. Galarza M. Evidence of the subcommissural organ in humans and its association with hydrocephalus, Neurosurgical Review. 2002;25(4):205-15.
58. Grondona JM, et al. Ependymal denudation, aqueductal obliteration and hydrocephalus after a single injection of neuroaminidase into the lateral ventricle of adult rats, J Neuropathol Exp Neurol. 2006;55(9):999-1008.
59. Vio K, et al. Hydrocephalus induced by immunological blockage of the subcommissural organ-Reissner's fiber (RF) complex by maternal transfer of anti- RF antibodies, Exp. Brain Research. 2000;135:41-52.
60. Woolam DHM. The circumventricular organs of the brain: Their possible role as sites for future neurosurgery, Annals of the Royal College of Surgeons of England. 1982;64:310–17.
61. Tretjakov D. Die zentralen Sinnesorgane bei Petromyzon Arch f. mikr. Anat, Bd. 1913;53.
62. Kolmer, W. Das “Saggitalorgan der Wirheltire, Zeitschirft Anat. Entwickl. Gesch 1921;652-171.
63. Agdhur E. Uber ein zentrales Sinnesorgan (?) bei den Vertebraten. Zischr. Anat. Entwickelungesch. 1922;66:223-30.
64. Vigh B. Actual problems of the cerebrospinal fluid-contacting neurons, Microscopy Research & Technique. 1998;41(1):57–83.
65. Vigh-Teichmann L, Vigh B. The system of cerbrospinal fluid-contacting neurons, Arch. Histol. Jap. 1983;46(4):427–68.
66. Blackshaw S, Snyder SH. Encephalopsin: A novel mammalian extraretinal opsin discretely localized in the brain J. Neurosci. 1999;19:3681-3690.
67. Nakane Y, et al. A mammalian neural tissue opsin (Opsin 5) is a deep brain photoreceptor in birds, Proc Natl Acad Sci. 2010;107(34):15264-15268.
68. Vigh B, Manzano MJ. Nonvisual photoreceptors of the deep brain, pinea organs and retina, Histol. Histopathol. 2002;17(2):555-90.
69. Kohno K. Electron microscope study of Reissner’s fiber, Z. Zellforsch. 1969;94:565-573.
70. Agajanian G. Raphe origin of serotonergic nerve terminals in the cerebral ventricles, Brain Research. 1978;88:223-31.
71. Mikkelsen JD, et al. Central innervation of the rat ependyma and subcommissural organ with special reference to ascending serotonergic projections from the raphe nucleus, Journal of Comparative Neurology. 1997;384(4):556–68.
72. Klein, M.O. et. al. Periaqueductal gray opioid receptors determine behavioral selection from maternal to predatory behavior in lactating rats, Behavioral Brain Research, 2014;62-72.
73. Jean-Stephane J, et al. Windows of the brain: Towards a developmental biology of circumventricular and other neurohemal organs, Seminars in Cell & Developmental Biology. 2007;18(4):512-24.
74. Craig AD. Interoception: The sense of the physiological condition of the body. Current Opin Neurobiol. 2003;13(4):500-5.
75. Mohseni M, et al. (eds.).Quantum Effects in Biology, Cambridge University Press; 2014.
76. Wineland D. Nobel Lecture: Superposition, entanglement, and raising Schrödinger’s cat, Rev. Mod. Rev. Mod. Phys. 2013;85:1103-1111.
77. Bernard T. Heaven Lies Within Us, New York: Rider and Company; 1940.

78. Wile L. Reissner's Fiber, Quanta and Consciousness, Journal of Consciousness Exploration and Research. 2012;3(8):1006-1017.
79. Sargent PE. The optic reflex apparatus of vertebrates for short-circuit transmission of motor reflexes through Reissner's fiber: Its morphology, ontogeny, phylogeny and function. Part I. The fish-like vertebrates, Bulletin of the Museum of Comparative Zoology at Harvard University. 1905;45:1-256.
80. Rakic P, Sidman R. Subcommissural organ and adjacent ependyma: Autoradiographic study of their origin in the mouse brain, The American Journal of Anatomy. 1968;122:317–36.
81. Einstein A. Ideas and Opinions New York: Crown Publishers; 1953.
82. Gokhman D, et al. Reconstructing the DNA Methylation Maps of the Neandertal and the Denisovan, Science. 2014;344(6183):523-527.
83. Greyson B. The Physio-Kundalini Syndrome and Mental Disease, Journal of Transpersonal Psychology. 1993;25(1):43–58.
84. Buckley P. Mystical experience and schizophrenia, Schizopren Bull. 1981;7(3):516-21.
85. Vilkov GA, et al. Detection of neurotropic properties of the serum inschizophrenics, Neurosci. Behav Physiol. 1984;14(6):441-3.
86. De Vernejoul P et al. Study of acupuncture meridians using radioactive tracers, Bulletin de L'Academie Nationale de Medicine, 169(7), pp. 1071–75. (references to Bong Han, Kim J. 1963. Democratic People's Republic of Korea Academy of Sciences No. 5; 1965. Proceedings Academy Kungrak DPRK.No. 2. Pongyang, Korea: Medical Sciences Press; 1985.
87. Lee BC. Novel anatomic structures in the brain and spinal cord of the rabbit that may belong to the Bonghan system of potential acupuncture meridians, Journal of Acupuncture Meridian Studies. 2008;1(1):29-35.
88. Vera A, et al. SCO-spondin from embryonic cerebrospinal fluid is required for neurogenesis during early brain development, Fron. Cell Neurosci. 2013 ;7:80.
DOI: 10.3389/fncel.2013.00080
89. Hafez DM, et al. F-spondin gene transfer improves memory performance and reduces amyloid-β levels in mice, Neuroscience. 2012;223:465-72.
90. Ho A, Sudhof TC. Binding of F-spondin to amyloid-beta precursor protein: A candidate amyloid-beta precursor protein ligand that modulates amyloid-beta precursor protein cleavage, Proc Natl Acad Sci USA. 2004;101(8):2548-53.

Biography of author(s)



Lawrence Wile
Chaikin-Wile Foundation, 8 Winged Foot Blvd., Bloomfield CT, 06002, USA.

He received a B.S. in physics from Union College (1971), an M.D. from the University of Connecticut School of Medicine (1976), a post-doctoral fellowship from Yale University School of Medicine (1979) and an M.A. in philosophy from the University of Massachusetts (1991). He is a Diplomate of the American Board of Psychiatry and Neurology. He is currently serving as the President of the Chaikin-Wile Foundation and is leading a multidisciplinary team at Boston University and the University of Connecticut dedicated to the exploration of Reissner's fiber. He is the author of the Jaynes Legacy: Shining New Light Through the Cracks of the Bicameral Mind.

© Copyright (2021): Author(s). The licensee is the publisher (Book Publisher International).

DISCLAIMER
This chapter is an extended version of the article published by the same author(s) in the following journal.
Journal of Consciousness Studies, 23(3-4):191-211(21),2016.

Chapter 7
Print ISBN: 978-93-90516-93-3, eBook ISBN: 978-93-90516-94-0

Influence of Climatic Changes on the Distribution of Geographical Spaces from the Floating Ivory Coast: Case of the Family of Acanthaceae

Ebah Estelle Asseh[1*], Emma Ake-Assi[1,2,3] and Kouao Jean Koffi[4]

DOI: 10.9734/bpi/rrab/v5/4991D

ABSTRACT

The object of this study is to estimate the diversity and the influence of the climate changes on spatial of the species of the family of Acanthaceae in Ivory Coast. Fluctuations in climatic variables such as precipitation and temperature are likely to affect biodiversity and the geographical distribution of habitats favourable to species. The principle of «Maximum Entropy» was used to model the habitats of *Dicliptera alternans* Lindau, *Elytraria ivorensis* Dokosi and of *Whitfieldia lateritia* Hook. under the current and future climatic conditions (horizon 2050/scénario A2). These species were selected on the one hand on the basis of number of occurrences and on the other hand by taking account of their endemic nature. The contact of presence of the species was collected and combined with the bioclimatic data extracted the database www.worldbioclim.org. The surface of the potentially favorable zones of distribution or not, was calculated for each species. The document retrieval revealed a total of 106 species left again between 39 kinds and of which 16% are endemic at the Ivory Coast. 7 species are register on the local list of the endangered species and rare of the Ivory Coast of Aké-Assi L. Les values of the AUC of about 0.90 indicate excellent quality of the models generated for the distribution of the model species by 2050, the results reveal that the «Seasonal variation of the temperature (Bio 4) » is the environmental variable which contributed the most to the prediction of the model of the species *Dicliptera alternans* Lindau, *Elytraria ivorensis* Dokosi and *Whitfieldia lateritia* Hook. The A2 scenario shows by a 2050 reduction of the surface of the potentially favorable zones of the species will des *Dicliptera alternans* Lindau, *Elytraria ivorensis* Dokosi and *Whitfieldia lateritia* Hook respectively of 67.66%, 3.35% and of 1.17%. In spite of these changes observed, the results show that the protected areas in Ivory Coast remain refuges under the current and future environmental conditions for the species of the family of Acanthaceae. This study confirms the impact proven of the climate changes on the spatiotemporelle distribution of the species. This work will help to better guide decision making in terms of conservation and management of protected areas as biodiversity refuges.

Keywords: Biological diversity; climate change; A2 Scenario; acanthaceae; Ivory Coast.

1. INTRODUCTION

Tropical forests are characterized by a very important biological richness with varied structures and floristic compositions. At the African level, this flora develops in various ecosystems, which leads to a very diversified flora and vegetation [1]. Tropical forests are the subject of several studies to better understand the role they could play in sustainable development, climate change, and floristic biodiversity [2-4]. With threats to biodiversity, some of these species are becoming increasingly rare in their natural habitats. In addition to anthropogenic threats, climate change now appears to be an additional threat to plant and animal populations [5]. Fluctuations in climatic variables such as

[1]*Laboratoire de Botanique, UFR Biosciences, Université Félix Houphouët-Boigny, Cocody-Abidjan, Côte d'Ivoire.*
[2]*Centre National de Floristique, 22 BP 582 Abidjan 22, Côte d'Ivoire.*
[3]*Institut Botanique Aké-Assi d'Andokoi (IBAAN), 08 BP 172 Abidjan 08, Côte d'Ivoire.*
[4]*Nangui Abrogoua, UFR-SN, 02 BP 801 Abidjan 02, Côte d'Ivoire.*
**Corresponding author: E-mail: assehebah@yahoo.fr;*

precipitation and temperature are likely to affect biodiversity and the geographical distribution of habitats favourable to species [6]. Côte d'Ivoire, like African countries, is experiencing the impacts of climate change on biodiversity. This has been confirmed by several research studies [7-10]. These authors confirm that Côte d'Ivoire has experienced significant variations in climatic conditions. They also predict an increase in temperature and a decrease in rainfall over most of the country. To date, there are few studies on the impact of climate change on the potential distribution of plant species. Some studies deal with the impact of current climate change on the distribution of Epiphytes [11], des Rubiaceae [12], endemic species [13] in Côte d'Ivoire and the species consumed by chimpanzees in the Park national of Taï [14]. Ose relating to the impact of future climatic conditions on the distribution of species belonging to the same taxon or taxa of a family have yet to be documented.

The Acanthaceae are one of the richest families of species in the world with about 3500 species grouped in 229 genus [15]. It consists of many species with very colourful flowers. Acanthaceae have long been appreciated for extreme diversity of pollen forms [16,17]. In Ivory Coast, this family is classified among the richest families of ornamental plants [18] belonging to the natural flora. In addition, species of this family are used as medicinal, ornamental and food plants [19]. Present throughout the Ivorian territory, species of the Acanthaceae family tend to disappear from their natural environment because of the strong anthropogenic disturbances of the natural forest environment [20]. It is in this already constraining context that climate change is compounded by the impact of climate change on biological diversity and the geographical distribution of habitats favourable to local species [6]. It is therefore necessary to assess the likely effects of climate change on the geographical distribution of these local species. However, studies that have assessed the potential impact of climate change on the geographical distribution of favourable areas for species in Côte d'Ivoire have yet to be documented. Thus, the objective of this study is to contribute to the knowledge of the impact of climate change on the geographical distribution of species in Côte d'Ivoire through the family Acanthaceae. Specifically, it will be a question of identifying the diversity (species richness and endemism), modelling the geographical distribution of the favourable areas of these species and evaluating the potential effect of climate change by 2050 on the extent of these areas and their geographical distribution.

2. MATERIALS AND METHODS

2.1 Cadre D'étude

This study was conducted in Côte d'Ivoire. Located in West Africa, Côte d'Ivoire is part of a square whose coasts are located between 4°30 and 10°30 North latitude and between 2°30 and 8°30 West longitude. The territory is bounded to the south by the Atlantic Ocean, to the north by Mali and Burkina Faso, to the east by Ghana and to the west by Liberia and Guinea. Its surface area is about 322,500 Km2. The study covers four climatic zones: sub-equatorial climate; humid tropical climate; tropical climate; and mountain climate. The vegetation of Côte d'Ivoire is subdivided into Domains and Sectors based mainly on the dominant plant formations in the landscape. These are as follows: the Guinean Domain, comprising, from South to North: the coastal sector; the ombrophilic sector; the mesophilic sector; the mountain sector; and the Sudanese Domain, comprising, from South to North: the Sub-Sudanese sector; the Sudanese sector. The Guinean domain is dominated by dense humid forest consisting of 2 main types of dense humid forest: evergreen or ombrophilic dense humid forest and semi-deciduous or mesophilic dense humid forest. As for the Sudanese domain, it is made up of open forests and savannas. However, in the face of the galloping degradation of the natural environment in Côte d'Ivoire, the protected areas represented by the 188 classified forests, the eight national parks and the six natural reserves are now the last remnants of the dense Ivorian forest (Sangne et al., 2015).

2.2 Collecting Data

The coordinates of presence of each species were collected in the herbarium of the Centre National de Floristique (C.N.F) of the University Félix Houphouët-Boigny, the herbarium of the Institut Botanique Aké-Assi d'Andokoi (IBAAN), the books, the data of the Global Biodiversity Information Facility (GBIF) on the website www.gbif.org. and of the SIG-IVOIRE were consulted. For some crops

that do not have geographical coordinates, the "Gazetiers", was used to identify their geographical coordinates. All this data was compiled and resulted in a list of species names corrected with the occurrences To establish the potential distribution models, we used nineteen (19) Bioclimatic variables (Table 1) downloaded from the Worldclim database version 1.4 via www.worldbioclim.org with a resolution of 2.5 arc minutes at ground level. The climatic conditions cover the current (1950-2000) and future (2000-2050) period. For future climate projections, there are several scenarios from the Special Report of the Intergovernmental Panel on Climate Change (IPCC, 2001) on Emission Scenarios (SRES). In this study, for all models, projections made for 2050 were used under the A2 emission scenario. This scenario was preferably used as it predicts a situation considered more likely for Africa by 2050 [21]. Indeed, the A2 emission scenario describes a very heterogeneous world, a population with a high growth rate and a low level of technology and development.

2.3 Analysis of Data

2.3.1 Biological diversity

The different biological types and phytogeographical distribution of Acanthaceae have been recorded with reference to the flora of Côte d'Ivoire [22-24]. The coefficient G / E (ratio of the number of genera to the number of species) was used to estimate the floristic diversity. A low value (close to 1) of this coefficient reflects the high diversity of the flora [12].

2.3.2 Model species

For this study, three species were used as models. They were chosen taking into account, on the one hand, that they have a number of occurrences ≥ 10 and, on the other hand, their special status. Indeed according to Gnagbo [1], the number of occurrences greater than ten (10) allows a robustness of the model. So *Whitfieldia lateritia* Hook. (49 occurrences), *Dicliptera alternans* Lindau (species classified as rare according to Aké-Assi, 1998) with 10 points of occurrence, and *Elytraria ivorensis* Dokosi (the only Acanthaceae endemic to Côte d'Ivoire) with 14 points of occurrence, have been retained.

2.3.3 Modeling and model validation

The modeling was based on the Maximum Entropy Model (MaxEnt). It is a program that allows the spatial distribution of a species to be estimated and predicted based on data on the presence of the species in relation to environmental conditions in the area of interest [25].

The nineteen current and future bioclimatic variables were tested for correlation. This test, which highlighted the existing links between the variables, enabled us to select the least correlated variables (correlation coefficient lower in absolute value, at 0.8). For Elith et al (2011), a high correlation between variables leads to bias in the predictions.

Modelling was done by introducing into the MaxEnt processing algorithm the geographic coordinates of occurrence of each species recorded in a CSV file [26]. Subsequently, current (1950-2000) and future (2050 horizon) bioclimatic variables were integrated into the same algorithm. In addition, a Jackknife test was conducted on the bioclimatic variables considered to determine which ones contributed most to the modelling [27]. Model performance was also evaluated using AUC statistics (Area Under the Curve) [25]. For a model generated by MaxEnt, Araújo et al. [28] recommend an interpretation of the AUC according to Table 2.

Arcgis10.1. software to map the current geographical distribution of the species' favourable habitats and those of the future was used. The network map.

The map of the national network of protected areas (national parks and reserves) of Côte d'Ivoire was superimposed on the potential distribution maps of each model species from MaxEnt.

The different habitat levels of our model species were classified based on the logistic probability thresholds of occurrence generated by the models. The logistic probability of occurrence of a species is considered as an indicator of habitat quality for the species. It varies between 0 and 1 [27]. For a

probability value below 0.229, the habitat is said to be "potentially unfavourable". Habitat is said to be "potentially favourable" for values above 0.229. The extent of each habitat level (area and percentage), both under current and future conditions, was estimated in order to assess the gain or loss in the potentially favourable area of the species at the country scale (Côte d'Ivoire). This estimation was carried out using the "spatial analyst" tool of the ArcGis software in its version 10.1.

Rates of change (Tc) from currently favourable to potentially unfavourable habitats in the future and vice versa were estimated for each model using the following formula [29]:

$$Tc= ((A2-A1)/A1) \times 100$$

In this study, A1 and A2 are respectively the initial (current climatic condition) and final (future climatic condition) area of presence of the species Positive values of Tc indicate a gain in habitat area while negative values correspond to a loss of area*

Table 1. Bioclimatic variables used for the Maxent test [30]

Bioclimatic variable	Name
Bio 1	Average annual temperature
Bio 2	Average diurnal deviation (Maximum temperature - Minimum temperature; monthly average
Bio 3	Isothermality (Bio1/Bio7)
Bio 4	Seasonality of temperature (coefficient of variation)
Bio 5	Maximum temperature of the hottest period
Bio 6	Minimum temperature of the coldest period
Bio 7	Annual temperature difference (Bio5-Bio6)
Bio 8	Average temperature of the wettest trimester
Bio 9	Average temperature of the driest trimester
Bio 10	Average temperature of the warmest trimester
Bio 11	Average temperature of the coldest trimester
Bio 12	Annual rainfall
Bio 13	Precipitation during the wettest period
Bio 14	Precipitation in the driest period
Bio 15	Seasonality of rainfall (coefficient of variation)
Bio 16	Precipitation in the wettest quarter
Bio 17	Precipitation in the driest quarter
Bio 18	Precipitation in the hottest quarter
Bio 19	Precipitation in the coldest trimester

Table 2. Validity of the Maxent test according to AUC values [28]

Values	Interpretations
0,90<AUC<1,00	Excellent
0,80<AUC<0,90	Good
0,70<AUC<0,80	Acceptable
0,60<AUC<0,70	Wrong
0,50<AUC<0,60	Invalid

3. RESULTS

3.2 Floristic Diversity

A total of 106 species grouped in 39 genera were inventoried. The genus most represented in species are the genus Justicia Retz (12.96%); Thunbergia Retz. (7.40%); Barleria (5.55%); Hygrophila (5.55%) and Asystasia (5.55%), Fig. 1. Nanophanerophytes (np) with 62% of the species are the most represented biomorphological type. Nanophanerophyte lianas (Lnp), pyrophytic hemicryptophytic

nanophanerophytes (npHpy) and terophytes (Th) are the least represented with 3% of the species each. (Fig. 2). Data indicate that 42% of the species of the family Acanthaceae belong to Guineo-Congolese (GC). Species endemic to the forest block in West Africa and Guineo-Congolese Sudano-Zambézian (GC-SZ) species account for 13% and 19% respectively (Fig. 3). In this database, 15 species (1%) are endemic to Côte d'Ivoire among which, Elytraria ivorensis Dokosi is the only Acanthaceae species endemic to Côte d'Ivoire (GCi) (Table 3). In this family nine species are listed in the IUCN Red List [31] and seven in the local list of threatened and rare Ivorian species of Aké-Assi [32], Table 4. The generic coefficient of the Acanthaceae family given by the number of genera over the number of species is 0.37 or 37%.

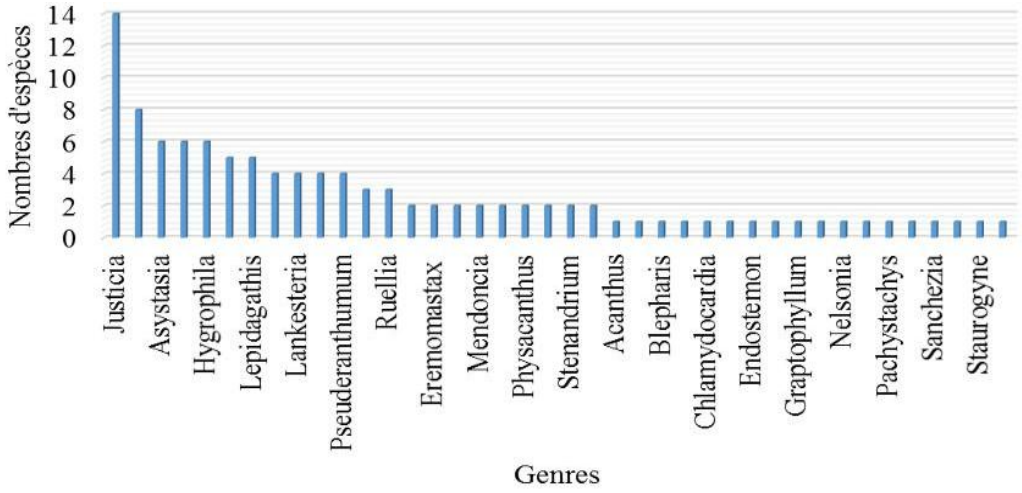


Fig. 1. Histogram of the specific richness by genus of the family Acanthaceae recorded in Ivory Coast

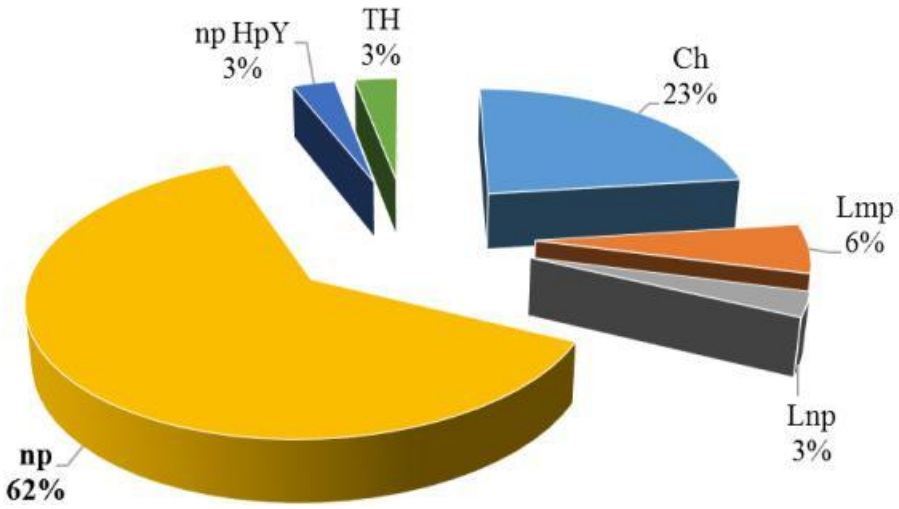


Fig. 2. Spectrum of biomorphological types of the species surveyed

Legend: mp: microphanerophytes; np: nanophanerophytes; Lmp: lianaectic microphanerophytes; Ch: chamephytes; Th: therophytes, Lnp: lianaectic nanophanerophytes; Lmp: lianaectic microphanerophytes; npHpy: hemicryptophytic nanophanerophytes. Pyrophytic

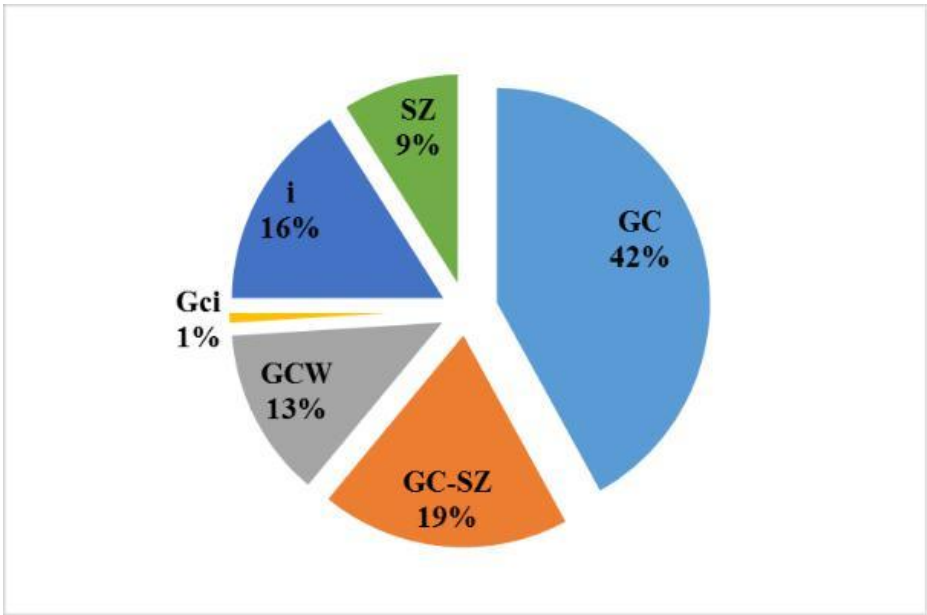


Fig. 3. Spectra of chorological affinities of all inventoried species
GC: Taxon of the Guineo-Congolese region; GCW: endemic species of the forest block in western Togo; GCI: endemic species of Côte d'Ivoire); SZ: Taxon of the Sudano-Zambézian region; GC-SZ: Taxon of the transition zone between the Guineo-Congolese and Sudano-Zambézian regions; i: Introduced exotic taxon

3.3 Contribution of Modelling Variables and Model Validation

Correlation analysis was used to select the least correlated (r < 0.80) environmental variables to run the models (Table 5). These are Bio 1 (Mean annual temperature), Bio 2 (Mean diurnal variation: maximum temperature - minimum temperature; monthly average), Bio 3 (Isothermality (Bio1/Bio7)* 100), Bio 13 (Precipitation of the wettest period) Bio 15 (Seasonality of precipitation: Coefficient of variation) Bio 16 (Precipitation in the wettest quarter), Bio 18 (Precipitation in the warmest quarter) and Bio 19 (Precipitation in the coldest quarter) under current climatic conditions. With regard to future climatic conditions these are the variables Bio 4 (Seasonality of temperature (Coefficient of variation)), Bio 6 (Minimum temperature of the coldest period) and Bio 13 (Precipitation of the wettest period). The table also presents the contribution of the variables to the construction of the different models. The model generated CUA values ranging from 0.918 to 0.988 for current climate conditions and from 0.923 to 0.985 for future climate conditions in 2050 under scenario A2 (Table 6).

3.4 Current and Future Distribution of Habitats Favourable to Model Species

3.4.1 Distribution of *Dicliptera alternans* lindau

The results of the various simulations show that, for the period 1950-2000, the potentially favourable area of the species covers approximately 12.12% of the territory, i.e. 39105.89 km². This potentially favourable area is mainly located in the south-western part of the Taï National Park (PNT). It also reaches the centre of Côte d'Ivoire in the vicinity of the Marahoué National Park. (Fig. 4). The potentially unfavourable zone occupies 283357.11 km² or 87.8% of the national territory (Table 7). For the execution of the model, the bioclimatic variable "Isothermality (Bio1/Bio7) * 100" is the variable with the highest contribution (81.5%) in the construction of the D. alternans distribution model (Fig. 5).

With bioclimatic projections to 2050, D. alternans will lose nearly 67.66% (Table 7) of the areas currently favourable to it. This area will only be observed in the Taï National Park (TNP), Fig. 4: "Seasonality of temperature, (Bio 4)" is the main variable that contributes most to the prediction of the potentially favourable area of the species (Fig. 5).

3.4.2 Distribution of *Elytraria ivorensis* dokosi

The habitat analysis gives an area of 63309.07 km² (or 19.63%) as a potentially favourable distribution area for the species under current conditions (Table 7). This area is located in the southwest within and around the Taï National Park and in the southeast of Côte d'Ivoire in the vicinity of the Ehotilés Islands Nature Reserve (Fig. 6). As for the area potentially unfavorable to the distribution of the species, it extends over 259153.93 km² or 80.36% of the Ivorian territory. "Average diurnal variation (Bio 2)", (72.4%) and "Isothermality (Bio1/Bio7) * 100, (Bio 3)", (24.7) are the bioclimatic variables that have contributed most to the construction of the distribution model of E. ivorensis for current climatic conditions (Fig. 7). By 2050, the A2 scenario projects an increase of nearly 0.82% in the areas potentially unfavourable to the species through the loss of areas potentially favourable to the species (Table 7). It increases from 259153.93km² (current climate conditions) to 261277.18km² (future climate conditions). The scenario also predicts that the areas potentially favourable to E. ivorensis in the southeast will no longer be favourable (Fig. 6). The environmental variables "Seasonality of temperature (Bio 4)" (84.4%) and "Seasonality of temperature (Bio 5)" (84.4%) are also included in the scenario. «Minimum temperature of the coldest period (Bio 6 ", (15.5%) a better contribution to model building (Fig. 7).

3.4.3 Distribution of *Whitfieldia lateritia* hook

The current distribution map (1950-2000) shows that the potentially favourable distribution areas for W. lateritia Hook. in the national territory are the Taï National Park (TNP) in the south-west and in the west at Mount Nimba (Fig. 8). These areas represent 12972.91 km2 or 4% coverage (Table 7). The "Precipitation of the warmest quarter (Bio 18)", (32.5); "Precipitation of the coldest quarter (19), (27.7%), "Precipitation of the wettest period (Bio 13)", (24.6%) and "Isothermality (Bio 3)", (11.6%) are the bioclimatic variables that participated most in the prediction of the areas of occupation of the species (Fig. 9). With regard to future projections (period 2000-2050), the areas potentially favourable to the species will experience a slight decrease in their coverage of the order of 1.17% (Table 7). However, the location of these favourable areas remains the same. The variables best predicting the distribution of W. lateritia are "Seasonality of the temperature (Bio 4)", (41.9%); "Precipitation in the wettest period (Bio 13)", (28.5%) and "Precipitation in the coldest quarter (Bio 19)", (25.4%), (Fig. 9).

Table 3. Numbers of endemic species

Nº	species	Number of samples	
		Gci GCW	GCW-S
1	*Brachystephanus jaundensis subsp. nimbae* (Heine) I. Darbysh.	3	
2	*Elytraria ivorensis* Dokosi	14	
3	*Heteradelphia paulojaegeria* Heine	3	
4	*Hygrophila odora* (Nees) T. Anderson	7	
5	*Justicia grandis* (T. Anderson) Vollesen	7	
6	*Justicia guineensis* (Heine) W.D. Hawth.	5	5
7	*Lankesteria hispida* (Willd.) T. Anderson	1	
8	*Mendoncia combretoides* (A. Chev.) Benoist	11	
9	*Rhinacanthus virens var. Obtusifolius (Nees) Milne-Redhead*	11	11
10	*Rungia eriostachya Hua*	2	
11	*Staurogyne capitata E. A. Bruce*	23	
12	*Sclerochiton vogelii (Nees) T. Anderson*	15	
13	*Stenandrium buntingii (S. Moore) Vollesen*	15	15
14	*Thomandersia anachoreta Heine*	13	13
15	*Whitfieldia colorata C.B. Clarke ex Stapf*	12	12
16	*Whitfieldia lateritia Hook.*	49	49

Legend: GCi: species endemic to Côte d'Ivoire; GCW: endemic species of the West African forest block; GCW-S: endemic species of the West African forest block, mainly in the eastern limit of the Sassandra river located in the hygrophilic forests in the west of Côte d'Ivoire

Table 4. List of species with special status

Lists	species	Statute
UICN (2016)	*Stenandrium guineense* (Nees) Vollesen	LC
	Brillantaisia lamium (Nees) Benth.	LC
	Dicliptera eliotti C. B. Cl.	LC
	Hygrophila senegalensis (Nees) T. Anders.	LC
	Phaulopsis imbricata (Forssk.) Sweet	LC
	Physacanthus nematosiphon (Lindau) Rendle & Britten	DD
	Rhinacanthus virens (Nees) Milne-Redh. var. virens	LC
	Ruellia primuloides (T. Anderson ex Benth.) Heine	LC
	Sclerochiton vogelii (Nees) T. Anderson	LC
Aké-Assi (1998)	*Brillantaisia madagascariensis* T. Anderson ex Lindau	PRE
	Chlamydocardia buettneri Lindau	PDE
	Dicliptera alternans Lindau	PRE
	Dicliptera laxispica Lindau	PRE
	Heteradelphia paulojaegeria Heine	PRE
	Sclerochiton vogelii (Nees) T. Anderson	PRE
	Thunbergia affinis S.Moore	PRE

Legend: LC: Minor Concern; DD: Insufficient Data EPP: Rare, Endangered and Rare Plants; EDP: Extinct and Endangered Plants

Table 5. Selected environmental variables and their contribution to the model

		***Dicliptera alternans* Lindau**		***Elytraria ivorensis* Dokosi**		***Whitfieldia_lateritia_ Hook.***	
Code	**Variable name**	**Current**	**horizon 2050**	**Current**	**horizon 2050**	**Current**	**horizon 2050**
Bio 1	Température moyenne annuelle	0	0	0	0	0,4	0
Bio 2	Ecart diurne moyen (température maximale – Température minimale; moyenne mensuelle)	0,4		**72,4**		0,2	
Bio 3	Isothermalité (Bio1/Bio7) * 100	81,5		24,7		11,6	
Bio 4	Saisonnalité de la température (Coefficient de variation)		78,8		84,4		41,9
Bio 6	Température minimale de la période la plus froide		10,5		15,5		4,3
Bio 13	Précipitations de la période la plus humide	0,2	1,3	0	0	24,6	28,5
Bio 15	Saisonnalité des précipitations (Coefficient de variation)	0		1,8		0	
Bio 16	Précipitations du trimestre le plus humide	2,9		0		3	
Bio 18	Précipitations du trimestre le plus chaud	12,2		0		32,5	
Bio 19	Précipitations du trimestre le plus froid	2,8	9,3	1,1	0	27,7	25,4

Table 6. Different CUA values from modeling results

Taxons	AUC (present condition)	AUC (horizon 2050)
Dicliptera alternans Lindau	0,988	0,985
Elytraria ivorensis Dokosi	0,918	0,923
Whitfieldia_lateritia_ Hook.	0,981	0,959

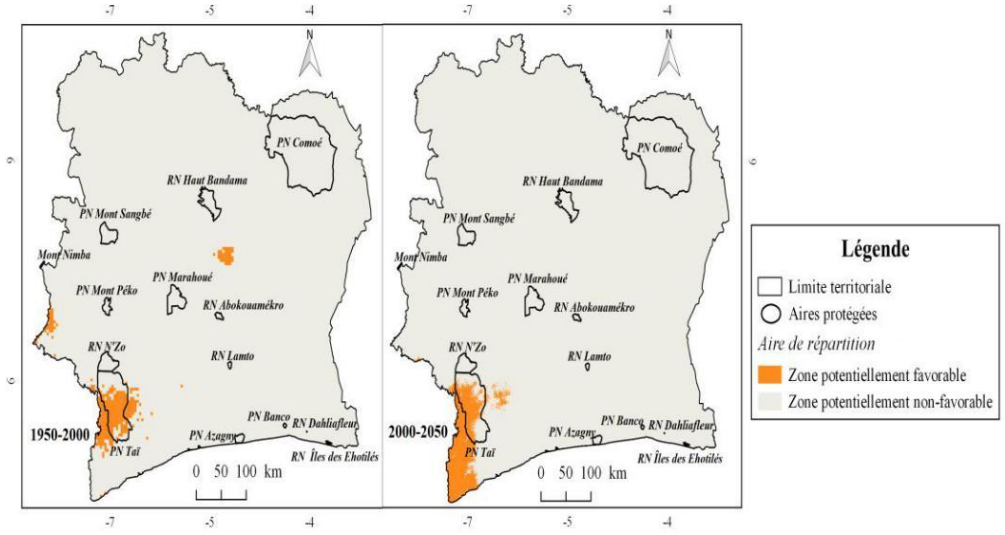
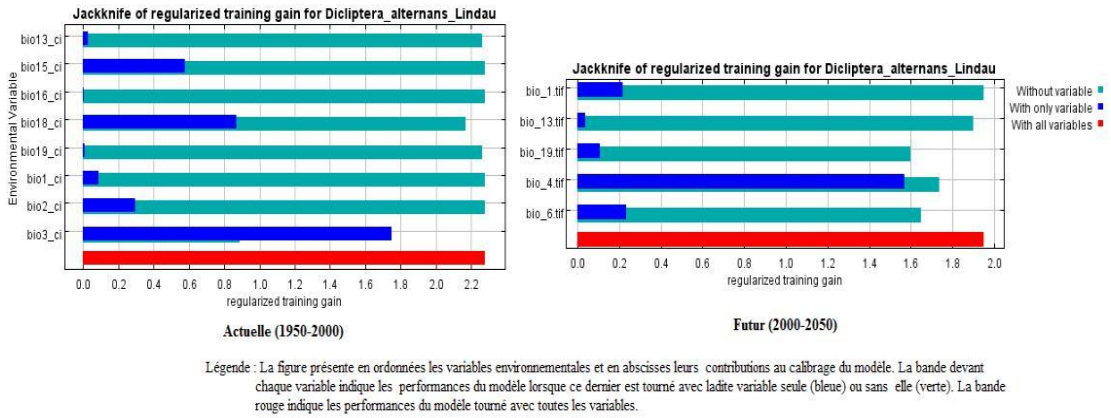


Fig. 4. present and future potential distribution of *Dicliptera alternans* Lindau in Côte d'Ivoire

Table 7. Dynamics of potential distribution areas of model species

Species	Period	Potentially favourable area		Change rate (%)	Potential area unfavourable		Change rate (%)
		Area (km²)	% of coverage		Area (km²)	% of coverage	
Dicliptera alternans Lindau	Actuel	39105,89	12,12		283357,11	87,8	
	2050	12646,94	3,92	-67,66	309816,06	96,07	+9,34
Elytraria ivorensis Dokosi	Actuel	63309,07	19,63		259153,93	80,367	
	2050	61185,82	18,97	-3,35	261277,18	81,02	+0,82
Whitfieldia lateritia Hook	Actuel	12972,91	4,02		309490,09	95,97	
	2050	12820,91	3,97	-1,17	309642,09	96,024	+0,04

A minus sign (-) indicates a loss of favourable habitat and a plus sign (+) indicates a gain (%): percentage



Légende : La figure présente en ordonnées les variables environnementales et en abscisses leurs contributions au calibrage du modèle. La bande devant chaque variable indique les performances du modèle lorsque ce dernier est tourné avec ladite variable seule (bleue) ou sans elle (verte). La bande rouge indique les performances du modèle tourné avec toutes les variables.

Fig. 5. Result of the Jackniffe test on the bioclimatic variables of the model

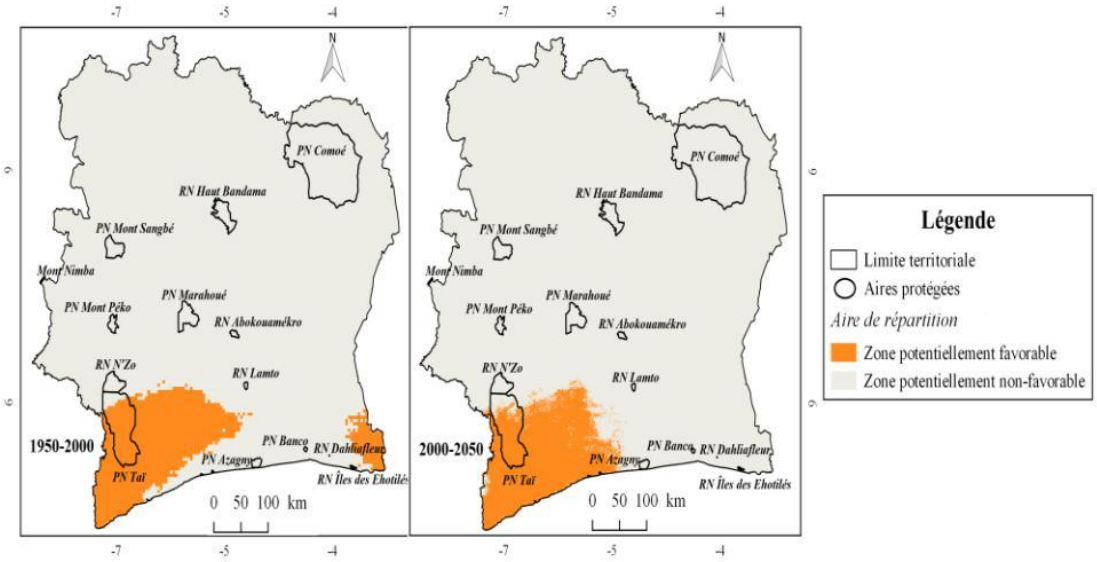
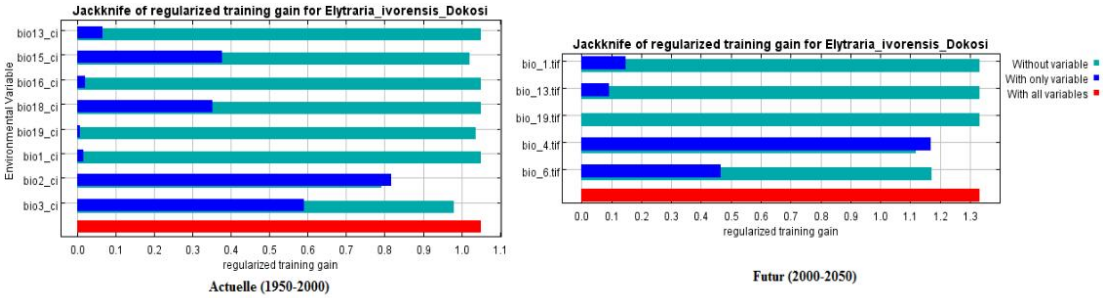


Fig. 6. Potential current and future distribution of Elytraria ivorensis Dokosi in Côte d'Ivoire



Légende : La figure présente en ordonnées les variables environnementales et en abscisses leurs contributions au calibrage du modèle. La bande devant chaque variable indique les performances du modèle lorsque ce dernier est tourné avec ladite variable seule (bleue) ou sans elle (verte). La bande rouge indique les performances du modèle tourné avec toutes les variables.

Fig. 7. Result of the Jackniffe test on the bioclimatic variables of the model

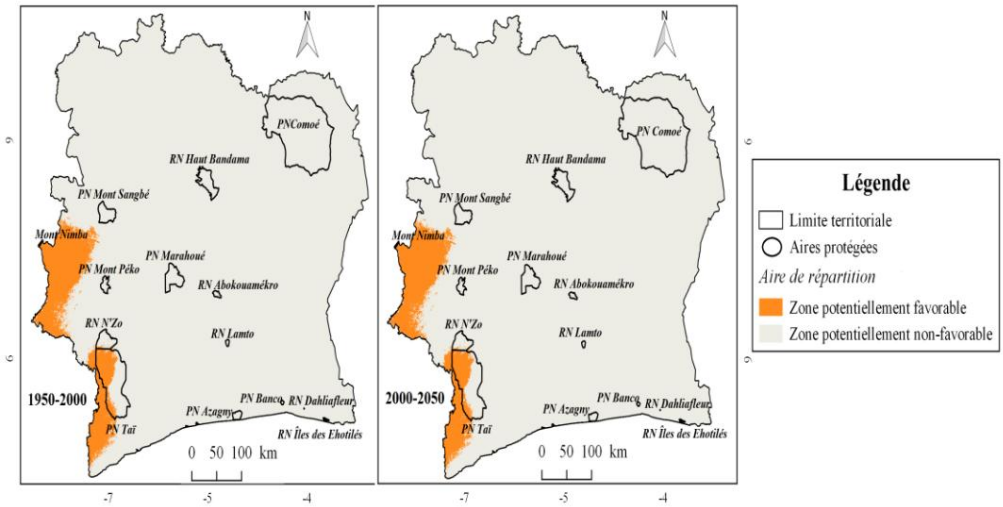


Fig. 8. Current and future potential distribution of Whitfieldia lateritia Hook in Côte d'Ivoire



Légende : La figure présente en ordonnées les variables environnementales et en abscisses leurs contributions au calibrage du modèle. La bande devant chaque variable indique les performances du modèle lorsque ce dernier est tourné avec ladite variable seule (bleue) ou sans elle (verte). La bande rouge indique les performances du modèle tourné avec toutes les variables.

Fig. 9. Result of Jackniffe's test on bioclimatic variables in the model

4. DISCUSSION

4.1 Diversity of Acanthaceae in Côte d'Ivoire

The number of species (106) and genera (39) obtained in this study is different from that of Aké-Assi [24] (85 species and 35 genera). This difference is due, on the one hand, to the fact that we have taken into account all the species without distinction as to whether or not they belong to the natural flora of Côte d'Ivoire. On the other hand, the compilation of information from several data sources. By comparing the taxonomic richness of our flora with that of Central Africa, we note that it is richer than ours with 310 species and 48 genera listed obtained by Koffi [33]. This can be explained by the fact that Koffi's [33] work was carried out in a vast phytogeographic area including the Democratic Republic of Congo, Rwanda and the Burundi and represents the diversity of Acanthaceae of a region while the present study was carried out in an area more restricted. The dominance of phanerophytes and Guineo-Congolese (GC) type species in the floristic procession would indicate that a large part of the Acanthaceae has a preference for the ombrophilous sector. The relatively high proportion of introduced species (16%) confirms the classification of this family among the richest in ornamental plants by Aké-Assi [18] in Côte d'Ivoire. The author also points out that introduced plants occupy a prominent place in the Ivorian ornamental flora. The diversity observed within this family is reflected in the presence of species with special status and endemic species.

4.2 Qualities of the Model and Contribution of Environmental Variables

UC values of the order of 0.90 indicate excellent quality of the models generated for the distribution of model species. This indicates the robustness of the modelling performed and attests to the excellent performance of the MaxEnt algorithm in predicting the different habitats of *Dicliptera alternans* Lindau, *Elytraria ivorensis* Dokosi and *Whitfieldia lateritia* Hook. However, it is important to remain vigilant with these results, since ecological factors and edaphic variables were not integrated into the model. Despite these difficulties, Shmueli [34] states that they are minor as long as the objective remains the prediction and not the explanation of a species' niche. Furthermore, Fandohan et al. [35] indicate that the MaxEnt model does not constitute a problem when the study area covers a reduced scale and the reference area for testing and calibrating the model is limited to climatic conditions where the species is under the influence of the same climatic factors. The results show that the "Seasonality of temperature (Bio 4) "is the environmental variable that contributed most to the model prediction of the *Dicliptera alternans* Lindau, *Elytraria ivorensis* Dokosi and *Whitfieldia lateritia* Hook species. by 2050.

This suggests that the future distribution of these species will be mainly influenced by temperature variations.

4.3 Potential Species Distribution Area

Selon les résultats de nos travaux, les zones de distribution potentiellement favorables de nos espèces modèles actuelles et futur se retrouve à l'intérieur et aux alentours des aires protégées. Cela montre que le territoire national, à travers son réseau d'aires protégées, présente des conditions actuelles et futures favorables à la distribution des Acanthaceae. Ils confirment de ce fait que les aires protégées constituent des refuges qui favorisent encore la conservation de la biodiversité [36] notamment des espèces endémiques et internationalement ou localement en danger face aux effets du changement climatique à l'horizon 2050. Cependant, les projections à l'horizon 2050 indiquent une régression de la superficie des zones potentiellement favorables des espèces *Dicliptera alternans* Lindau, *Elytraria ivorensis* Dokosi et *Whitfieldia lateritia* Hook. Cette régression s'expliquerait principalement par la projection de la hausse de la température et de la baisse rainfall levels in Côte d'Ivoire (Goula et al., 2006) [10]. These results are in line with numerous studies that highlight the regression of areas favourable to the distribution of certain species [37,38,26,35]. Thus, the hypothesis that climate change could modify the distribution range of species seems to be confirmed. According to WWF [39], the rise in temperature and the fall in precipitation are the result of the increase in concentrations of anthropogenic greenhouse gas (GHG) emissions, which are strongly linked to the decline in dense evergreen forests. This regression of forests observed in Côte d'Ivoire, like in tropical countries, due to anthropogenic pressures, leads to a loss of natural habitats favourable to species as observed in our results. The regression of potentially favourable areas of distribution of the species is done by conversion into potentially unfavourable areas inducing an increase in their surface area. Scenario A2 focuses on economic development based on a high-growth population with a low level of technology and development. Thus, to meet the needs of this growing population, there will be an increasing conversion of forests to agricultural areas. This would be one of the reasons for the increase in areas not favourable to our species. Moreover, Davis et al. [40] already estimated that 60,000 plant species will disappear by 2050 if current trends continue. These observations show that our species would probably see a regression in the size of their favourable distribution area by 2050 under the combined effect of the degradation of forest areas and climatic variations.

5. CONCLUSION

This study is a contribution to the improvement of the knowledge of biodiversity and the assessment of the impact of climate change in Côte d'Ivoire through the family Acanthaceae. To this end, it focused on the analysis of the diversity and potential distribution of some species of Acanthaceae present in the herbaria of Côte d'Ivoire. The richness of this family is estimated at 106 species grouped in 39 genera among which 16 endemic species and 7 species on the local list of endangered and rare Ivorian species of Aké-Assi [32]. Acanthaceae are generally Guineo-Congolese type nanophanerophytes with a relatively high proportion of introduced species. The low value of the generic coefficient indicates good diversity within this family. Like most countries in the tropical zone, Côte d'Ivoire is subject to climate change. This study carried out on the distribution of species specifically on those of Acanthaceae, illustrates this phenomenon well and gives an idea of its impact on the spatial distribution of species. This study shows that by 2050, the effect of climate change coupled with the degradation of the vegetation cover will lead to a significant decrease in the surface area of potentially favourable distribution areas for the species *Dicliptera alternans* Lindau*, Elytraria ivorensis* Dokosi and *Whitfieldia lateritia* Hook. respectively by 67.66% (i.e. a loss of 26459.04 km^2), 3.35% (i.e. a loss of 2120.85 km^2) and 1.17% (i.e. a loss of 151.78 km^2). On the other hand, increases in potentially relatively unfavourable areas will be observed for these species. However, despite these observed changes, the results show that environmental conditions in Côte d'Ivoire will remain favourable for the distribution of Acanthaceae, especially in the protected area network. This work thus makes it possible to assess the importance of the spatiotemporal distribution of species of local populations and their vulnerability to the effects of climate change. This work will help to better guide decision making in terms of conservation and management of protected areas as biodiversity refuges.

ACKNOWLEDGEMENTS

The authors would like to thank the National Floristic Centre (CNF), the Aké-Assi Botanical Institute of Andokoi (IBAAN), the Swiss Centre for Scientific Research (CSRS) and the Global Biodiversity Information Facility (GBIF) for making our database available. They also thank all the readers who contributed to the improvement of the scientific quality of the manuscript.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Gnagbo A. Diversité, distribution et utilisations des épiphytes vasculaires des strates inférieures des forêts côtières de Côte D'Ivoire : Cas du Parc National d'Azagny. Thèse de doctorat en écologie végétale, UFR Biosciences, Université Félix Houfouët Boigny, Abidjan, Côte d'Ivoire. 2015 ;216.
2. Lewis SL, Lopez-Gonzalez G, Sonké B et al. Increasing carbon storage in intact African tropical forests. Nature. 2009 ;457(7232)1003–1006.
3. Djuikouo MNK, Doucet JL, Nguembou CK, Lewis SL, Sonké B. nDiversity and aboveground biomass in three tropical forest types in the Dja Biosphere Reserve, Cameroon. African Journal of Ecology. 2010 ;48(4):1053–1063.
4. Ifo SA, Moutsambote JM, Koubouana F, Yoka J, Ndzai SF, Bouetou-Kadilamio LNO, Mbemba M. Tree species diversity, richness, and similarity in intact and degraded forest in the tropical rainforest of the Congo Basin: case of the forest of Likouala in the Republic of Congo. International Journal of Forestry Research; 2016.
5. Sala E, Ballesteros E, Starr RM. Rapid decline of Nassau grouper spawning aggregations in Belize: Fishery management and conservation needs. Fisheries. 2001;26(10):23-30. DOI:10.1577/1548-8446(2001)026<0023:RDONGS>2.0.CO; 2
6. IPCC. Climate change. Synthesis Report. Contribution of Working Groups I, II & III to the Fourth Assessment Report of the Intergovernmental Panel on Climate Change. Geneva Cambridge University Press Cambridge: UK and New York-USA. 2007;339-378. DOI: 10.1017/CBO9780511546013.
7. Kouakou E, Goula BTA, Kouassi AM. Analyze of climate variability and change impacts on hydro-climate parameters: case study of Côte d'Ivoire. International Journal of Scientific & Engineering Research. 2012;3(2):1-8.
8. Sorokoby VM, Saley MB, Kouamé KF, Djagoua EMV, Affian K, Biemi J.Variabilité spatio-temporelle des paramètres climatiques et son incidence sur le tarrissement dans les bassins versants du Bô et Debo (département de Soubré au Sud-Ouest de la Côte d'Ivoire). International Journal of Innovation and Applied Studies. 2013;2(3):287-299.
9. Kouamé YM, Soro GE, Kouakou KE, Kouadio ZA, N'Diaye MEH, Goula BTA, Issiaka S. Scenarios des changements climatiques pour les précipitations et les températures en Afrique subsaharienne tropicale humide : cas du bassin versant de Davo, Côte d'Ivoire. Larhyss Journal. 2014;18:197-213.
10. Oga YMS, Adja M, Yapi AF, Kpan JG, Baka D, Yao KT, Biémi J. Projection de la variabilité climatique à l'horizon 2050 dans la zone côtière au Sud-Est de la Côte d'Ivoire (d'Abidjan à Aboisso). Larhyss Journal. 2016 ;25:67-81.
11. Gnagbo A, Kpangui KB, Adou YCY. Distribution des épiphytes de Côte d'Ivoire: Effets des zones phytogéographiques et des variations pluviométriques. Afrique Science. 2015;11(1):175-186.
12. Tuo FN, Kouao JK, Kouassi FA, Kone M, Bakayoko A, Bogaert J. Etude de la diversité, de l'endémisme et de la distribution spatiale des Rubiaceae de Côte d'Ivoire. Int. J. Biol. Chem. Sci. 2017;11(2):777-797. DOI: https://dx.doi.org/10.4314/ijbcs.v11i2.20
13. Vroh BTA, Adou YCY, Kpangui KB, Goné BZB, Kouamé D, Koffi JK. Comparing suitable habitat models to predict rare and endemic plant species distributions: What are the limits of the niche

of *Cola lorougnonis* (Malvaceae) in Côte d'Ivoire? Environment and Natural Resources Research. 2016;6(3):1-17.
DOI:10.5539/enrr.v6n3p1

14. Condé A. Impact des variations climatiques sur la distribution des principales espèces végétales dont les fruits sont consommés par les chimpanzés dans Le Parc National De Taï, Côte D'Ivoire. Mémoire de Master de Biodiversité et Valorisation des Écosystèmes de l'Université Félix Houphouët-Boigny, Côte D'Ivoire. 2017;56.
15. APG IV. An update of the Angiosperm Phylogeny Group classification for the orders and families of flowering plants.APG IV ; 2016.
16. Scotland RW, Vollesen K. Classification of Acanthaceae. Kew Bull. 2000;55:513–589.
17. Tripp EA, McDade LA. A rich fossil record yields calibrated phylogeny for Acanthaceae (Lamiales) and evidence for marked biases in timing and directionality of intercontinental disjunctions. Systematic Biology. 2014;63(5):660-684.
18. Aké-Assi E. Plantes à potentialité décorative de la flore du sud de la Côte d'Ivoire : études taxinomique, ethnobotanique et essai de domestication de *Thunbergia atacorensis* Akoegninou et Lisowski (Acanthaceae), une espèce nouvellement introduite. Thèse de Doctorat, UFR Biosciences, Université Félix HOUPHOUEËT-BOIGNY, Côte d'Ivoire. 2015;207.
19. Grubben GJH. Ressources végétales d'Afrique Tropicale 2. Légumes Fondation Prota Wageningen : Pays Bas/ Backhuys. 2004;737.
20. Aké-Assi E, Koffi N'G, Tetchi NA. Atlas de la biodiversite de l'afrique de l'Ouest (Tome III). Atlas: Côte d'Ivoire. 2010;324-325.
21. Williams JW, Jackson ST, Kutsbach JE. Projected distribution of novel and disappearing climates by 2100 AD. Proc. Natl. Acad. Sci. U.S.A. 2007;104:5738-5742.
22. Aké-Assi L. Flore de la Côte d'Ivoire. Etude descriptive et biogéographique, avec quelques notes ethnobotaniques. Thèse Doctorat, Université Nationale d'Abidjan, Côte d'Ivoire. 1984;1206.
23. Aké-Assi L. Flore de la Côte d'Ivoire 1, catalogue, systématique, biogéographie et écologie. Conservatoire et Jardin Botanique de Genève: Suisse- Boisseria. 2001;57:396.
24. Aké-Assi L. Flore de la Côte d'Ivoire 2, catalogue, systématique, biogéographie et écologie. Conservatoire et Jardin Botanique de Genève: Suisse Boisseria. 2002;58:441.
25. Phillips SJ, Anderson RP, Schapire RE. Maximum entropy modeling of species geographic distributions. Ecol. Model. 2006;190:231-259.
DOI: 10.1016/j.ecolmodel.2005.03.026
26. Gbesso FHG, Tente BHA, Gouwakinnou NG, Sinsin BA. Influence des changements climatiques sur la distribution géographique de *Chrysophyllum albidum* G. Don (Sapotaceae) au Benin. Int. J. Biol. Chem. Sci. 2013;7(5):2007-2018.
DOI: http://dx.doi.org/10.4314/ijbcs.v7i5.18
27. Fandohan B, Gouwakinnou G, Fonton NH, Sinsin B, Liu J. Impact des changements climatiques sur la répartition géographique des aires favorables à la culture et à la conservation des fruitiers sous-utilisés cas du tamarinier au Bénin. *Biotechnol.* Agron. Soc. Environ. 2013;17(3): 450-462.
DOI: https://popups.uliege.be:443/1780-4507/index.php?id=10186.
28. Araújo MB, Pearson RG, Thuiller W, Erhard M. Validation of species climate impact models under climate change. Global Change Biology. 2005;11:1504-1513.
DOI: https://doi.org/10.1111/j.1365-2486.2005.01000.x
29. Toyi MS, Barima YSS, Mama A, André M, Bastin J-F, De Cannière C, Sinsin B, Bogaert J. Tree plantation will not compensate natural woody vegetation cover loss in the Atlantic Department of Southern Benin. Tropicultura. 2013;31(1):62-70.
30. Guarino L, Jarvis A, Hijmans RJ, Maxted N. Geographic Information Systems (GIS) and the Conservation and Use of Plant Genetic Resources. In: Managing Plant Genetic Diversity, Engels JMM, Ramanatha RV, Brown AHD, Jacson MT(éd). International Plant Genetic Resources Institute (IPGRI) Rome: Italie. 2002;387-404.
31. UICN. The IUCN Red List of Threatened Species. UICN ; 2016.
Available:www.iucnredlist.org
32. Aké-Assi L. Impact de l'exploitation forestière et du développement agricole sur la conservation de la biodiversité biologique en Côte d'Ivoire. Le Flamboyant. 1998;(46):20-21.

33. Koffi KJ. Analyse des structures spatiales des données de distribution phytogéographique des Acanthaceae en Afrique centrale. Thèse de Doctorat Université Libre de Bruxelles, Faculté des sciences, Ecole Interfacultaire de Bioingenieurs, Service d'Ecologie du paysage et Systèmes de production végétale, Bruxelles. 2008;240.
34. Shmueli G. To explain or to predict? Statistical Science. 2010;25(3):289-310. DOI: 10.1214/10-STS330
35. Fandohan AB, Moutouama JK, Samadori S, Biaou H, Gouwakinnou GN, Adomou CA. Le réseau d'aires protégées Bénin-Togo assure-t-il la conservation de *Thunbergia atacorensis* (Acanthaceae)? Rev. CAMES. 2015;03(02).
36. Houinato M, Sinsin B, Lejoly J. Impact des feux de brousse sur la dynamique des communautés végétales dans la forêt de Bassila (Bénin). Acta Bot. Gall. 2001;48(3):237-251. DOI:10.1080/12538078.2001.10515891
37. Boko M, Niang I, Nyong A, Vogel C, Githeko A, Medany M, Osman-Elasha B, Tabo R, Yanda P. Africa. Climate change 2007: Impacts, adaptation and vulnerability. contribution of working group ii to the fourth assessment report of the intergovernmental panel on climate change. Parry ML, CanzianiOF, Palutikof JP, van der Linden PJ, Hanson CE (eds). Cambridge UK, Cambridge University Press: Cambridge. 2007;433-467.
38. Houessou GL, Lougbegnon OT, Gbesso GHF, Anagonou SEL, Sinsin B. Ethnobotanical study of the African star apple (*Chrysophyllum albidum* G. Don) in the Southern Benin (West Africa). Journal of Ethnobiology and Ethnomedicine. 2012;8:40. DOI: https://doi.org/10.1186/1746-4269-8-40
39. WWF (World wide fund for nature). Rapport des impacts du changement climatiques sur les espèces. WWF. 2011;21.
40. Davis SD, Droop SJM, Gregerson P, Henson, Leon CJ, Villa-Lobos JL, Synge LH, Zantovska J. Plants in Danger. What do we Know? IUCN: Gland Switzerland and Cambridge U.K. F. 1986; 520.

Biography of author(s)



Ebah Estelle Asseh
Laboratoire de Botanique, UFR Biosciences, Université Félix Houphouët-Boigny, Cocody-Abidjan, Côte d'Ivoire.

She is a holder of a unique PhD at the UFR Biosciences of the University Felix Houphouët-Boigny and defended on 04 December 2019. At the same time she was an instructor at the Botanical Laboratory and a volunteer at the Aké-Assi Botanical Institute of Andokoi (IBAAN). This is a foundation that works for the preservation of African flora in general and Ivorian in particular, by promoting and perpetuating the work of Professor Aké-Assi Laurent. The thesis work was carried out in the context of the development and management of heritage species in the context of conservation and sustainable use of biodiversity. They are part of the conservation of plants of interest to the populations and the enhancement of local plant biodiversity in the ornamental horticulture of Côte d'Ivoire. She is co-author of two publications on the floristic diversity, the level of knowledge of uses, the influence of climate change on the geographical distribution of certain species of the family Acanthaceae in Côte d'Ivoire. Her areas of interest are teaching, research, management and valorization of herbarium data, phytogeography, the socio-economic importance of biodiversity, monitoring and conservation of biodiversity from bioclimatic data as well as the establishment of priorities for biodiversity conservation through domestication.



Emma Ake-Assi
Laboratoire de Botanique, UFR Biosciences, Université Félix Houphouët-Boigny, Cocody-Abidjan, Côte d'Ivoire and Centre National de Floristique, 22 BP 582 Abidjan 22, Côte d'Ivoire and Institut Botanique Aké-Assi d'Andokoi (IBAAN), 08 BP 172 Abidjan 08, Côte d'Ivoire.

She is a Doctor of State in Natural Sciences in Botany, Lecturer and Teacher-Researcher at the UFR Biosciences of the University Félix Houphouët-Boigny. As an option, she mainly directs her research towards the Ivorian, or even African, ornamental flora, innovative work for their Laboratory. The domestication of the decorative species of the spontaneous flora being a means of safeguarding them; tests have been undertaken in this direction. Four lines of research emerge from my investigations: Biodiversity of decorative flora; valorization of the spontaneous decorative flora through Ethnobotany; Valorization of the collections of Herbarium; finally, Biodiversity fodder and urban development. At the academic level, the courses given from the Licence 1 to the Master 2 are about General Botany (Morphology, Cytology, Histology and Anatomy), Nomenclature, Systematics and Herbalism, in addition to the supervision of PhD students. As an Associate Researcher at the Centre National de Floristique (CNF), she is in-charge of the Pedagogical Research Unit related to the environment, society and sustainable development. The activities initiated in this field consist of training courses on the education of the population for a sustainable management of the plant heritage. At the same time, she is the Director of the Aké-Assi Botanical Institute of Andokoi (IBAAN). It is a foundation that works for the preservation of African flora in general and Ivorian flora in particular, through the promotion and perpetuation of the work of Professor Aké-Assi Laurent.



Kouao Jean Koffi
Nangui Abrogoua, UFR-SN, 02 BP 801 Abidjan 02, Côte d'Ivoire.

He was born on December 26, 1972 in Baffia (Aboisso) in Côte d'Ivoire. In 2008, he obtained his Unique Doctorate at Free University of Brussels in Belgium. He was recruited on March 02, 2009 as Teacher-Researcher at the Laboratory of Biology and Improvement of Plant Productions, UFR Natural Sciences at Nangui Abrogoua University to the rank of Assistant. In July 2012, he rose to the rank of Assistant Professor and since July 2018, he is a member of the Laboratory of Ecology and Sustainable Development (LEDD) and of the Research Unit: Ecology and Biodiversity; he is also a member of the Biodiversity and Valuation of Ecosystem Services (BioValSE) team. At the academic level, he teaches at the License and Master level and he also supervises doctoral students. Since 2012, he has directed his research activities on the theme "Contribution to the realization of the flora of Côte d'Ivoire". He is the author and co-author of some twenty scientific publications. He has focused his research on botanical knowledge, floristic diversity, spatial distribution, rare species, the exploitation of bamboo and its socio-economic importance, the production and exploitation of fodder plants, attempts to domesticate certain plants and biological diversity and influence of climate change on the geographic distribution of some Acanthaceae species in Côte d'Ivoire. His area of interest is teaching, research, botany, plant ecology, phytogeography, systematics, phytosociology, flora, conservation biology.

© Copyright (2021): Author(s). The licensee is the publisher (Book Publisher International).

DISCLAIMER
This chapter is an extended version of the article published by the same author(s) in the following journal.
International Journal of Biological and Chemical Sciences, 13(2): 676-692, 2019.

Chapter 8
Print ISBN: 978-93-90516-93-3, eBook ISBN: 978-93-90516-94-0

Tidal Influence on Nutrients Status and Phytoplankton Population of Okpoka Creek, Upper Bonny Estuary, Nigeria

O. A. Bubu-Davies[1*] and O. A. Ugwumba[2]

DOI: 10.9734/bpi/rrab/v5/3427D

ABSTRACT

Okpoka Creek of the Upper Bonny Estuary in the Niger Delta is a tidal creek receiving organic anthropogenic effluents from its environs. The study investigated the influence of tides (low and high) on the species composition, diversity, abundance, and distribution of phytoplankton. The surface water and phytoplankton samples were collected monthly from May 2004 to April 2006 at both tides from ten stations according to standard methods. Phytoplankton was identified microscopically. Species diversity was calculated using standard indices. Data analyses were done using analysis of variance, Duncan multiple range, and descriptive statistics. Phosphate and ammonia exceeded international acceptable levels of 0.10 mg/L for natural water bodies indicating high nutrient status, organic matter, and potential pollutants. A total of 158 species of phytoplankton were identified. Diatoms dominated the phytoplankton (62.9%). Diversity indices of diatoms were 1.5±0.03 (Margalef) and 0.8±0.01(Shannon). Pollution-indicator species such as *Navicula microcephala, Nitzschia sigma, Synedra ulna* (diatoms), *Cladophora glomerata* (green alga), *Euglena acus* (euglenoid), *Anabeana spiroides* (blue-green alga) and *Ceratium furca* (dinoflagellate) were recorded at either only low, high or both tides. Concerted environmental surveillance on Upper Bonny Estuary is advocated to reduce the inflow of pollutants from the Bonny Estuary into this Creek caused by tidal influence.

Keywords: Microalage; community; nutrients; aquatic pollution; tide; Okpoka Creek; Niger Delta.

1. INTRODUCTION

Estuaries are unique aquatic environments that have an additional source of buoyancy input derived from riverine freshwater inflow and an additional source of mechanical energy input from the tides (tidal stirring). Estuarine ecosystems are very favourable for algae and animal life. T hey play an important ecological role because they are natural habitants of water. T hey are widely used as an indicator of water pollution [1]. The Bonny Estuary is one of the several estuaries in the Niger Delta swamps through which the Lower Niger and Benue Rivers flow into the ocean. The estuary is richly endowed with abundant aquatic resources (creeks, distributaries, flood plains and mangrove swamps with fin/shell fish resources, etc.). The estuary, its creeks, and tributaries consist of rich collection of flora and fauna constituting a unique tropical biodiversity. The vegetation of Bonny Estuary is dominated by the red mangrove *Rhizophora racemosa* and *R. mangle* [2]. The mangrove provides nurseries and feeding grounds for commercially important species of finfish and shellfish. Despite its good environment for aquatic life forms, the area is prone to pollution resulting from industries located along the shore of Bonny Estuary which are concentrated in this zone and they discharge their effluents directly into the estuary. The flushing action of the tidal flows contributes to moving these pollutants down into the coastal zones. Okpoka Creek is a tidal tributary of the Upper Bonny Estuary. Tidal influence on this creek is twenty-four hours at interval of six hours of low and high tides alternatively.

[1]Department of Fisheries and Aquatic Environment, Rivers State University of Science and Technology, P.M.B. 5080, Port Harcourt, Nigeria.
[2]Hydrobiology and Fisheries Unit, Department of Zoology, University of Ibadan, Ibadan 900001, Nigeria.
**Corresponding author: E-mail: daviesonome@yahoo.com;*

Tides play a major role in the functioning of many coastal systems. They are responsible for obvious midterm (spring-neap cycles) and short-term (low-high water cycles) variations in the abiotic and biotic characteristics of these systems [3]. Tidal flushing is one of the main bottom-up factors controlling phytoplankton biomass in estuaries besides nutrients [4]. Quite a number of works had been conducted to evaluate tidal control of plants and animals communities and processes in different systems. These include tide-induced phytoplankton and microphytobenthos exchanges [5]; tidal influence on bacteria, microphytoplankton, and microzoo-plankton abundance [6]; tidal stirring and phytoplankton bloom dynamic in an estuary [7]; tidal influence on zonation and occurrence of resident and temporary zooplankton in Mundaka Estuary, Bay of Biscay [3]; and effects of tidal shallowing and deepening on phytoplankton production dynamics: a modeling study [8] and dancing with the tides: fluctuations of coastal phytoplankton orchestrated by different oscillatory modes of the tidal cycle [9].

Phytoplankton populations are highly dynamic, and in many environments they experience episodes of rapid bio-mass increase (blooms), either as recurrent seasonal events or as higher frequency phenomena [7]. According to Badsi [1], Rajesh et al. [10], Ananthan et al. [11], Tiwari and Chauhan [12], Tas and Gonulol [13] and Saravanakumar et al. [14], phytoplanktonic organism is one of the initial biological components from which the energy is transferred to higher organisms through food chain. The density and the diversity of phytoplankton are biological indicators for evaluating water quality and the degree of eutrophication [1,15,16].

There are many human activities going on around and in the creek such as slaughtering of animal, transportation (boating, navigation), fishing, and waste disposal. However, there has been no information on the influence of tide on the nutrients status and phytoplankton community of Okpoka Creek, a tidal tributary of Upper Bonny Estuary. In order to bridge the existing gap in knowledge of the biotic and abiotic features of this estuary, there is therefore the need to provide useful information on the tidal variations of water nutrients and phytoplankton population of this Creek. Based on this, the study thus evaluated the influence of low and high tides on nutrients, abundance, species composition, diversity, and distribution of the phytoplankton of Okpoka Creek.

2. MATERIALS AND METHODS

2.1 Study Area

Okpoka Creek is located between longitudes 7°00 E and 7°15 N and latitudes 4°28 E and 4°40 N (Fig. 1). The vegetation is dominated by nypa palm (*Nypa frutican*) and mangroves, red mangrove (*Rhizophora racemosa*), and white mangrove (*Avicennia nitida*). It passes through many communities, namely, Oginigba, Woji, and Azubiae. Many man's activities going on within and around this creek include dredging, fishing, boating, navigation, washing, disposal of excreta, bathing, and swimming, to mention but a few. This aquatic body receives effluent discharges from the many industries (Snig, Far East paints, RIVOC, General Agro, Michelin tyres, Coca Cola, Halliburton, Schlumberger, Acorn, etc.) and main abattoir house sited close to it. The stretch of the coastal front of the creek has very high population density without waste management facilities.

All the industries in the Trans-Amadi Industrial Layout and the riverine dwellers discharge their wastes directly to this creek.

2.2 Sampling Stations

A total of ten stations were chosen at least 500 metres apart along the main creek course. These stations were Station 1 (Oginigba), Station 2 (Trans-Amadi by Schlumberger), Station 3 (by main abattoir house), Station 4 (Azubiae), Station 5 (Woji), Station 6 (Okujagu), Station 7 (Okuru-ama), Station 8 (Ojimba), Station 9 (Oba-ama), and Station 10 (Kalio-ama). There are many industries sited close to the river shore discharging effluents into the creek. The dominant vegetation is nypa palm (*Nypa frutican*) followed by drying up red mangrove. Patches of water hyacinth were seen during the rainy season. Manual dredging of sand is constantly going on.

2.3 Physicochemical Parameters of the Water

The following parameters were measured in situ and in laboratory following standard methods [17]. One-litre clean containers were used to collect water samples for physicochemical parameters at each station. All the kegs containers were kept in ice-chest box for laboratory analyses. Nitrate was determined by the Brucine method [17]. Spectrophotometer (Spectronic 21D) was used to measure the nitrate at 410 nm wavelength. Sulphate determination was carried out by turbidimetric procedure [17]. This involved the use of spectrophotometer (Spectronic 21D). The concentration of sulphate (mg/L) was measured; thus,

$$\text{Sulphate} = \frac{\text{mg}SO_4 \times 1000}{\text{Volume of sample used (mL)}}. \tag{1}$$

Phosphate-in-water levels were determined by standard test [17]. It involved the use of spectrophotometer (Spectronic 21D). The concentration of phosphate (mg/L) was measured; thus,

$$\text{Phosphate} = \frac{\text{mg}PO_4 \times 1000}{\text{Volume of sample used (mL)}}. \tag{2}$$

Ammonia concentrations in water samples were determined by the indophenol or phenate (phenol-hypochlorite) method. It was spectrophotometrically measured at 630 nm wavelength with spectronic 21D [17]. The concentration of total ammonia in the samples was computed from the equation

$$\frac{C_1}{C_2} = \frac{A_1}{A_2}, \tag{3}$$

where A_1 = the absorbance of the total ammonia-nitrogen standard, A_2= the absorbance of the sample, and C_1=the concentration of the total ammonia-nitrogen standard. C_2= the concentration of total ammonia nitrogen in the sample (Boyd [18]).

2.4 Collection of Phytoplankton Samples and Analyses

Samplings were done at low and high tides. Surface water samples were collected using one, one-litre wide mouth, plastic container [17] at each station. The sample in the container was fixed with 10% formalin for phytoplankton identification and enumeration (standing crop estimation). In the laboratory, samples were allowed to stand for a minimum of 24 hours before decanting the supernatant. The supernatant was removed carefully until a 50 mL concentrated sample was properly shaken, and 1 mL of sub sample was collected from it and transferred into a Sedgwick-Rafter counting chamber using a stampel pipette. Identification and enumeration (standing crop estimation) was carried out under a binocular compound microscope with magnification 40×400. Three replicates of the subsamples were analysed. For each sample, each solitary cell or group of cells was counted as one unit except for the diatoms which were counted in a cell by cell base. Results were expressed in a number of organisms per mL of sample. The Sedgwick-Rafter counting chamber contains exactly 1 mL (50 mm long×20 mm wide×1 mm deep) and has a surface area of 1000 mm^2. The exact area viewed within the ocular micrometer grid is also known.

The following formula was used for the calculation of plankton density:

$$\text{Density of plankton (Number of plankters per mL)} = (T)\frac{1000}{\text{AN}} \times \frac{\text{Volume of concentrate mL}}{\text{Volume of sample (mL)}}, \tag{4}$$

where T= total number of plankters counted, A= area of grid in mm^2, N= number of grids employed, and 1,000=area of counting chamber in mm^2 (Boyd [18]).

Identification and characteristics of planktonic species were made by the descriptive keys by Needham and Needham [19]; Newell and Newell [20]; Patrick and Reimer [21], Han [22], Durans and Leveque [23], and Prescott [24]; and Kadiri [25] amongst others. Margalef species diversity index was estimated by this formula

$$H = \frac{S-1}{\ln N}, \tag{5}$$

where S= the number of species (or other taxonomic group) and N= total number of phytoplankters (Boyd [18]).

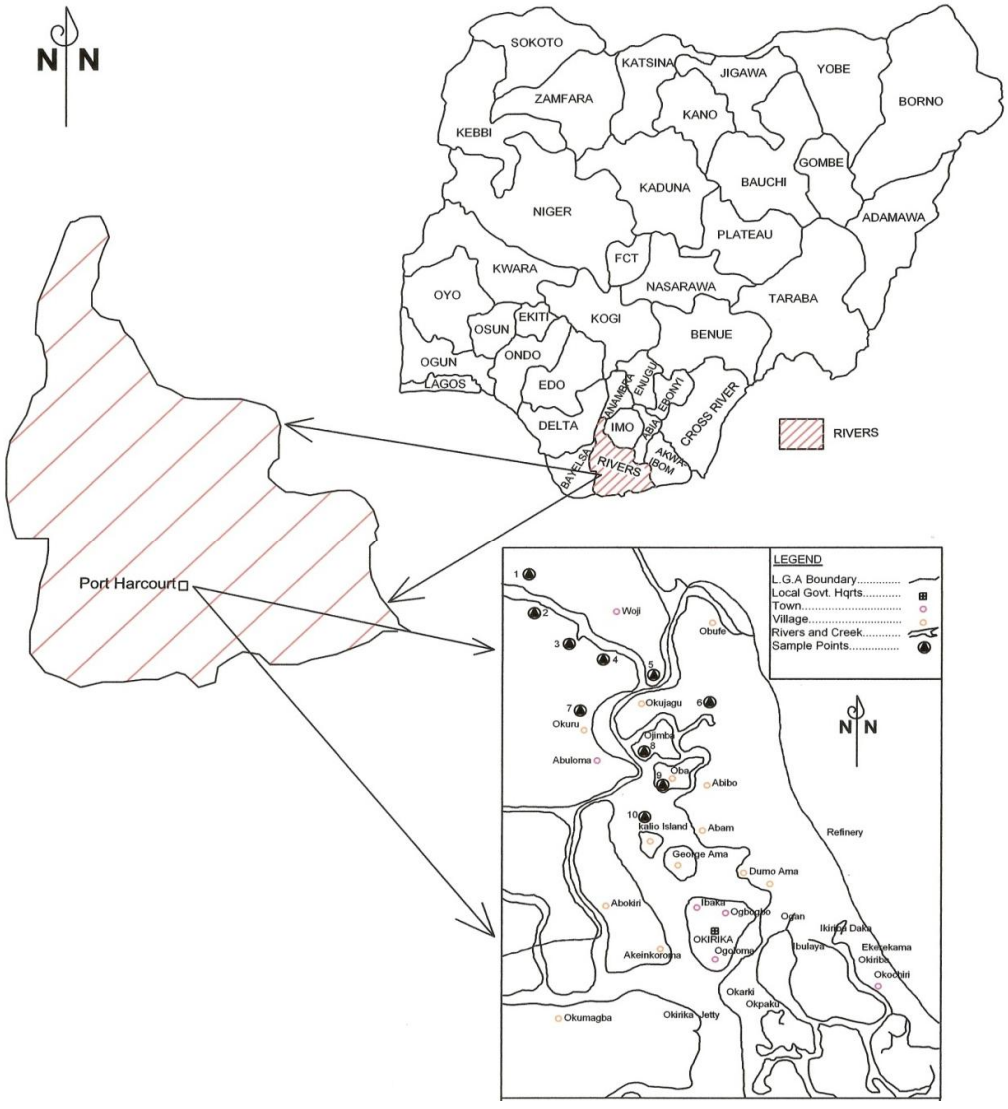


Fig. 1. Study area map

Table 1. Important values assigned to *i*th species abundance

Range	Importance Value
1-10	1
11-20	2
21-30	3
31-40	4
41-50	5
51-60	6
61-70	7
71-80	8
81-90	9
91-100	10
100-above	11

Index of dominance (C) was determined by the formula

$$C = \sum \left(\frac{ni}{N}\right)^2, \tag{6}$$

where ni= importance value for each species (number of individual, biomass, production, etc.) and N= total of importance values.

Importance values (Table 1) were assigned to the phyto-plankton species based on the contribution of each species to total net primary production [26] and also to pollution.

Index of similarity (S) in phytoplankton species diversity between the low and high tides was determined by index of similarity (S) between 2 adjacent samples [26] as follows:

$$S = \frac{2C}{A + B}, \tag{7}$$

where A= number of species in sample A,B= number of species in sample B and C number of species common in both samples

$$\text{Index of dissimilarity} = I - S. \tag{8}$$

Other indices of species diversity calculated are as follows.

(1) Shannon index of general diversity (H) was calculated thus

$$H = \sum \left(\frac{ni}{N}\right) \log \left(\frac{ni}{N}\right) \quad \text{or} \quad -\sum Pi \log Pi, \tag{9}$$

where ni= importance value for each species, N= total of importance values and Pi= importance ni probability for each species = ni/N.

(2) Evenness index (e) is

$$e = \frac{H}{\log_e S}, \tag{10}$$

where H= Shannon index and S =number of species.

3. DATA ANALYSES

SAS [27] was used to analyse data for analysis of variance (ANOVA), Duncan multiple range (DMR) and descriptive statistics.

Table 2. Variations of water nutrients in relation to tide in Okpoka Creek

Tide	Ammonia (mg/L)	Nitrate (mg/L)	Phosphate (mg/L)	Sulphate (mg/L)
Low	0.17±0.01[a]	0.68±0.02[a]	0.77±0.05[a]	586.11±36.42[a]
High	0.19±0.02[a]	0.48±0.04[b]	0.29±0.07[b]	459.09±33.89[a]

Means with the same letter in the same column are not significantly different (P> 0.05)

4. RESULTS AND DISCUSSION

4.1 Nutrients of Surface Water from Okpoka Creek

Generally, phytoplankton growth is limited by inorganic nutrients (phosphorous, nitrogen, silicon, iron, etc.). Nutrients status affects the composition of phytoplankton as its composition changes with the nutrient fluxes because individual taxa have different requirements. High tide ammonia level (0.19± 0.02 mg/L) was not significantly (P>0.05,DMR) higher than low tide ammonia (0.17 ± 0.01 mg/L) with a mean of 0.17±0.01 mg/L (Table 2). This possibly indicated anthropogenic inputs from the numerous industries and riverine dwellers located along the water course of Okpoka Creek at high tide. Higher concentration of ammonia at high tide favoured the observed diatoms, green algae, blue green algae, dinoflagellates, and xanthophytes especially the dominant diatoms at high tide. The lower concentration of ammonia at low tide favoured the other phytoplankton groups (chrysophytes and euglenoids). Ammonia is one of the nutrients required by phytoplankton for primary productivity. Others are nitrate, phosphorus, and sulphate [28]. Furthermore, ammonia is a source of nitrogen and contributes to the fertility of water since nitrogen is an essential plant nutrient. It is also one of the most important pollutants in aquatic environment because of its relative high toxic nature and its ubiquity in surface water systems [29]. Ammonia enters natural water systems from several sources including industrial wastes, sewage effluents, coal gasification and liquefaction conversion process plants, and agricultural discharge including feedlot runoff. The study ammonia exceeded the concentration of less than 0.1 mg/L found in natural waters [30]. This possibly indicates anthropogenic and domestic inputs. The mean ammonia concentration is also higher than the level of 0.02 mg/L unionized ammonia (NH3) required for the protection of aquatic life [31]. However, the recorded range of ammonia in this creek is within the range of 0.093mg/L to 2.65 mg/L reported by Chindah and Nduaguibe [32] and Obunwo et al. [33] in the Niger Delta.

Nitrate nitrogen and ammonia nitrogen determine community productive levels. The lower concentrations of sulphate, nitrate, and phosphate at high tide might signify low primary productivity. Tides cause a pattern of stabilizationde stabilization in circulation that results in the highest rates of primary production in many estuarine, coastal, oceanic, or frontal environments [34]. Destabilization period occurs during flood or ebb tides or during fast current movement. This results in a replenishment of nutrients to the photic zone from more nutrient-rich underlying waters. During and following the destabilization period, photosynthesis is decreased by a shortage of light due to increased turbidity and mixing of the phytoplankton to deeper waters. Circulation patterns are vital in establishing the balance between light levels and nutrient availability necessary to maintain high primary productivity rates in marine systems.

The higher concentration of nitrate, phosphate, and sulphate at low tide might be attributed to the higher abundance of chrysophytes and euglenoids at low tide. These nutrients indicated high primary productivity and that these phytoplankters are primary producers. At low tide, stabilization in circulation leads to increased rate of photosynthesis. Stabilization period occurs during slack tides or slow currents and results in increased rates of photosynthesis and nutrient uptake [34]. Nutrients availability especially phosphorus structures the algae community [35]. In addition, primary

productivity (phytoplankton productivity) is expected to be high at low tide (low water) as turbidity is low and solar and light penetrations are high.

Nitrate concentration (0.68±0.02mg/L) was high at low tide and low (0.48±0.04mg/L) at high tide with a mean of 0.64 ± 0.02mg/L. The highest nitrate recorded at low tide might be indicative of high human excrement and industrial discharges. Stabilization period occurs during slack tides or slow currents and results in increased rates of nutrient uptake [34]. This also might be the possible reason for this observation. Tidal effect was highly significant (P<0.001, DMR). The observed mean nitrate was below 100 mg/L expected to be found in natural surface water. Nitrogen is most often limiting in marine systems [36]. The reason being that molybdenum, phosphorus, or energy constraint can be limiting to nitrogen fixers, which makes for lower nitrogen fixation [37]. The range of nitrate recorded in this study was below the statutory limit of 25–50 mg/L given by the European Economic Community (EEC) [38] and 20 mg/L United State Environment Protection Agency (USEPA) [39]. Low tide phosphate level (0.77±0.05mg/L) was higher than that of high tide (0.29±0.07 mg/L) with a mean of 0.70±0.05mg/L. The effect of tide was significant (P < 0.05, DMR).

The higher phosphate at low tide could be due to high decomposition of organic matter and stabilization period. Tide influence on the sulphate concentrations was not significant (P>0.05). The recorded phosphate concentrations in this study were higher than the acceptable limit of 0.10 mg/L in flowing waters recommended by USEPA [39] and United States Geological Survey (USGS) [40]. This observation agrees with that of 0.43 to 3.52 mg/L of Chindah and Nduaguibe [32].

Low tide mean sulphate level was 586.11±36.42 mg/L (higher) and high tide level was 459.09±33.89 mg/L (lower) with a mean value of 560.05±28.87 mg/L. This could be attributed to the higher biological oxygen demand and stabilization period at low tide than at high tide. Oxidation of the organic materials and burning of fossil fuel used up oxygen, thereby exerting higher biological oxygen demand in the creek. The high sulphate level observed in this study is characteristic of brackish water. Ebere [41] reported that marine waters are known to contain relatively high concentrations of sulphate. McNeely et al. [30] observed 2650 mg/L sulphate in seawater. The recorded sulphate is below the sulphate values in seawater, that is, within the acceptable levels.

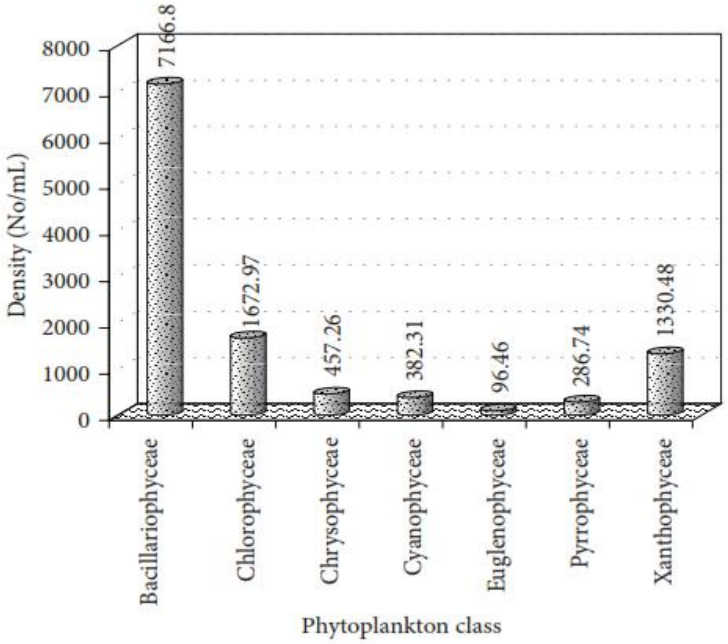


Fig. 2. Overall mean value of phytoplankton density in Okpoka Creek

4.2 Phytoplankton Community of Okpoka Creek

Phytoplankton are free-floating, and mostly microscopic plants, and they cannot swim; instead, they are transported by tides and currents. A total of forty-nine thousand four hundred and seventy nine (48,479) phytoplankton consisting of seven (7) families, seventy-nine (79 genera), and one hundred and fifty-eight (158) species were identified. These values were more than the values reported for studies on other waters of the Niger Delta. This could be due to environmental influence. The algae were Bacillariophyceae (diatoms), 80 species; Chlorophyceae (green algae), 44 species; Cyanophyceae (blue-green algae), 21 species; Euglenophyceae (euglenin), 6 species; Pyrrophyceae (dinoflagellates), 4 species; Xanthophyceae (xanthophytes), 2 species; and Chrysophyceae (chrysophytes), 1 species. The observed abundance of phytoplankton differs with that of Edoghotu [42] for Okpoka Creek though it agrees with that study in terms of diatoms being the most abundant phytoplankton. Other studies in the Niger Delta of Nigeria are those of Chindah, and Keremah [43], Chindah and Braide [44, 45] on the Bonny Estuary. Diatom dominance in this creek corresponded with the study of Badsi et al. [1] on Massa Estuary. Diatoms recorded the highest density (Fig. 2) and this indicated high productivity of Okpoka Creek according with Badsi et al. [1] Also, Moser et al. [46] stated that diatoms are sensitive to a wide range of limnological and environmental variables, and that their community structure may quickly respond to changing physical, chemical, and biological conditions in the environment. This might be the possible reason for the dominance of diatoms in Okpoka Creek. In addition, reasons for the varied tidal abundance, distribution, species composition, and diversity of diatoms could be ascribed to their resilient ability to withstand organic pollution. Furthermore, dominance of diatoms in terms of abundance and species composition indicates pollution [47]. This shows that diatoms are resilient to the increased anthropogenic inputs and high turbidity at high tide. The recorded abundance of the other phytoplankton classes explains this resilient ability of diatoms. Nevertheless, some algae inhibit the growth of others, for example, *Pyrmnesium parvum* suppresses other algae by production of allelopathic chemical [48]. Passy et al. [49] reported that diatoms distribution is associated with a downstream increase in nutrient level and organic pollution.

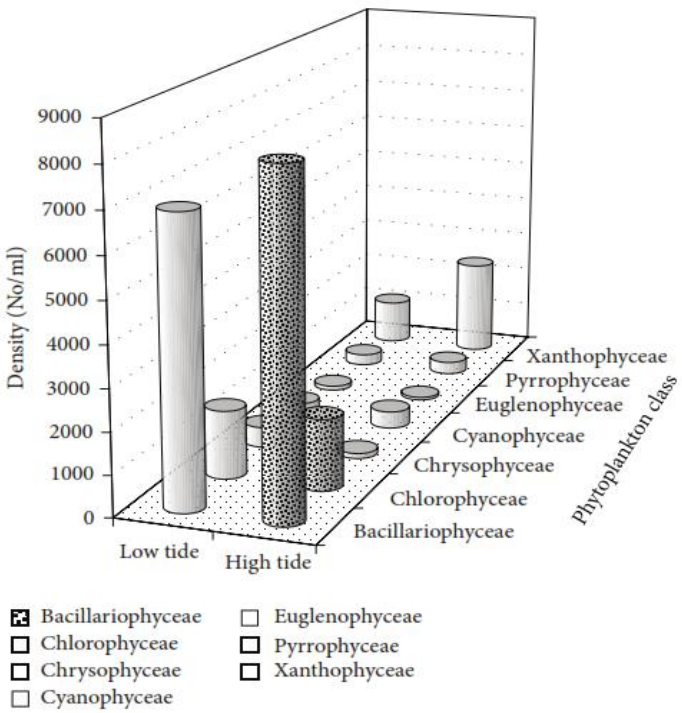


Fig. 3. Variations of phytoplankton density in relation to tide in Okpoka Creek

Chrysophytes and euglenoid flagellates recorded higher density at low tide (495.31±79.33 no/mL and 105.74±19.55 no/mL) than at high tide (124.25±33.99 no/mL and 68.63±17.78 no/mL) (Fig. 3). This could possibly be explained by the stabilization in circulation. The stabilization in circulation favoured these algae. The other observed algae recorded higher densities at high tide than at low tide. Tidal cycle is a major determinant of phytoplankton fluctuations at several different time scales [9]. A pattern of stabilization-destabilization in circulation results in the highest rates of primary production in many estuarine, coastal, oceanic, or frontal environments [34]. Stabilization period occurs during slack tides or slow currents and results in increased rates of photosynthesis and nutrient uptake. On the other hand, destabilization period is experienced during flood or ebb tides or during fast current movement results in a replenishment of nutrients to the photic zone from more nutrient-rich underlying waters. During and following the destabilization period, photosynthesis is decreased by a shortage of light due to increased turbidity and mixing of the phytoplankton to deeper waters [34]. Therefore, circulation patterns are vital in establishing the balance between light levels and nutrient availability necessary to maintain high primary productivity rates in marine systems. The higher phytoplankton biomass at low tide than at high tide was also reported by Okpuruka [50] of Elechi Creek in the Niger Delta. Okpuruka [50] reported that Elechi Creek of the Upper Bonny Estuary in the Niger Delta had higher phytoplankton diversity during spring tides than neap tides.

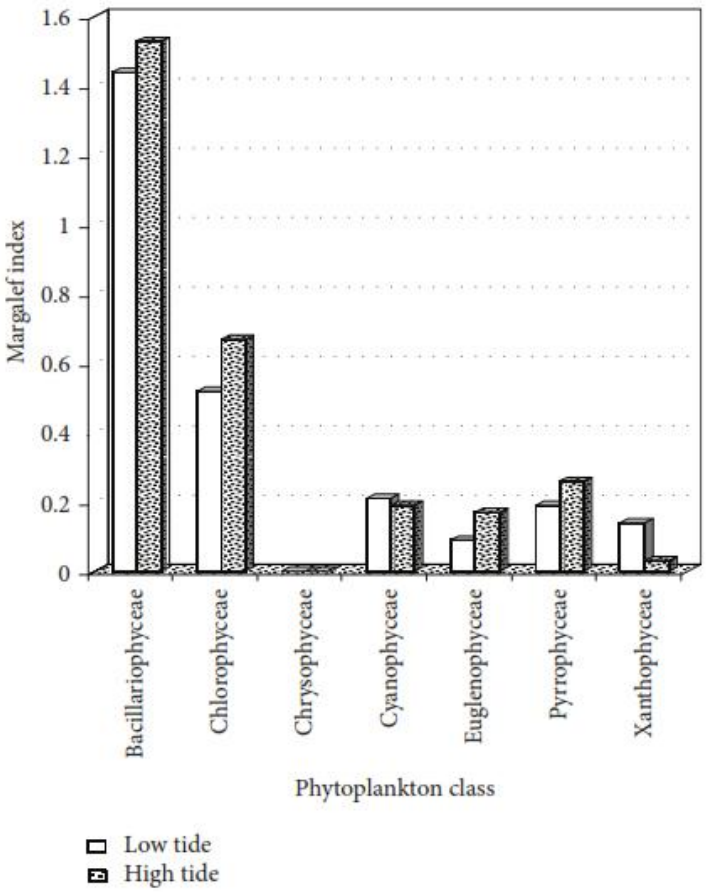


Fig. 4. Variation of phytoplankton Margalef index in relation to tide in Okpoka Creek

4.3 Bacillariophyceae (Diatoms)

Diatoms density ranged from 6908.92±394.37 no/mL (low tide) to 8140.29±729.63 no/mL (high tide) with a mean value of 7166.80±347.73 no/mL which was 62.91% total abundance of phytoplankton

(Figs. 2 and 3). High tide phytoplankton parameters [density (8140.29±729.63 no/mL), Margalef (1.53 ±0.05), Shannon (0.84±0.02), Evenness (0.42±0.01)] were higher except Dominance (0.15±0.01) than at low tide [density (6908.92±394.37 no/mL) Margalef (1.44±0.03), Shannon (0.77±0.01), and Evenness (0.40±0.01), and Dominance (0.18±0.01)] (Figs. 2, 4, 5, 6, and 7). Tidal effects were significant on all phytoplankton parameters except Margalef and Dominance indices ($P<0.01$, DMR).

The presence of diatoms at both tides indicated that species are euryhaline. High values of these parameters except Dominance index signified high diversity of diatoms at high tide. This observation could be attributed to tidal influence. At high tide, there is inflow of water from the sea into Okpoka Creek via the Upper Bonny Estuary that brings in phytoplanktonic organisms and other aquatic life. Phytoplankton are passively drifted microscopic plants, thus, some marine diatom species might moved into this creek. Tidal flushing is one of the main bottom-up factors controlling phytoplankton biomass in estuaries besides nutrients [4]. According to Villate [3], tides are responsible for obvious midterm (spring neap cycles) and short-term (low-high water cycles) variations in the abiotic and biotic characteristics of estuaries. The recorded higher dominance index at low tide might indicate that the observed diatom species were true species (permanent residents) of this creek. The Shannon index value below 3 up to 3 at both tides showed a low specif ic structure of this group. Based on principle, a low diversity is a feature of young settlements of species; while a high diversity indicates mature settlements, the low diversity index shows a weak internal structure of populations [1]. The lower abundance and species diversity of diatoms at low tide could be linked with the cycle of advection, settling, and resuspension that follow the suspended particles during the ebb, slack, and flood tidal stages. This cycle facilitates the retention of passive diatoms by physical trapping in the Okpoka Creek. In this creek, the pattern of abundance and species diversity of diatoms match the tidal cycles suggesting that diatoms are displaced along the creek within a determinate mass of water. This means that physical trapping and high water residence times serve to trap passively sinking particles, such as diatoms, without migrating behaviours in their optimum estuarine environments [4].

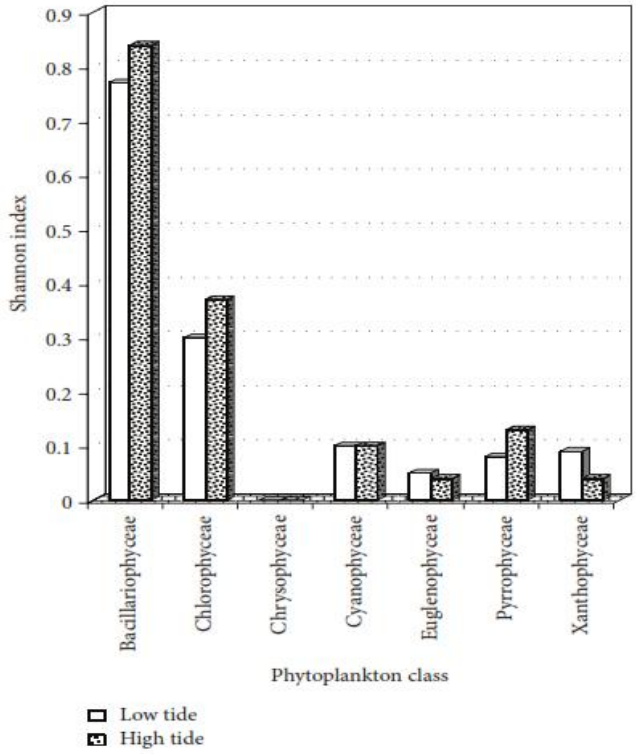


Fig. 5. Variation of phytoplankton Shannon index in relation to tide in Okpoka Creek

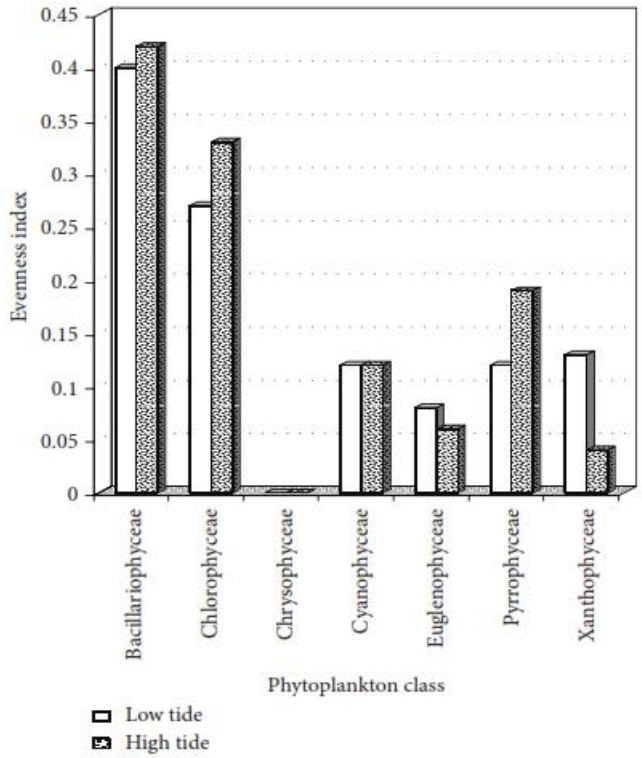


Fig. 6. Variation of phytoplankton evenness index in relation to tide in Okpoka Creek

There were 4 dominant genera and 10 prominent diatoms species out of the 80 observed species (Table 3). The species were *Melosira undulata* (26.03±16.05%), *Cyclotella operculata* (17.24± 1.63%), *M. pusilla* (17.66±0.96%), *Nitzschia sigma* (15.15±1.64%), *M. distans* (12.16±4.13%), *N. bilobata* (11.39±1.95%), *M. varians* (12.54±0.73%), *Navicula bacillum* (11.37±1.18%), *Cyclotella comta* (10.89±1.32%), and *Nitzschia filiforms* (11.32±0.85%). Tide had varied significant effects on these 10 dominant species ($P<0.05$, DMR). Percentage abundances of *C. comta*, *Nitzschia bilobata*, *N. sigma*, and *Navicula bacillum* were significantly higher at low tide than at high tide except *N. sigma* ($P<0.05$) while the opposite was observed for *C. operculata*, *M. varians*, and *M. distans*. Percentage abundances of *C. operculata* and *M. distans* were not significantly different ($P>0.05$). *N. filiforms*, *M. undulate*, and *M. pusilla* were absent at low tide. *Navicula gracilis*, *N. cuspidata*, *N. placentula*, *N. microcephala*, *N. bacillum*, *N. amphibola*, *Nitzschia sigma*, *N. bilobata*, *N. lanceolata*, *N. paradoxa*, *filiforms*, *N. longissima*, *Cymbella cuspidata*, *C. lata*, *Synedra ulna*, *Cyclotella meneghiniana*, *Tabellaria fenestrate*, *Cocconeis placentula*, and *Pinnularia major* are organic pollution indicator species and were present at both tides [51–56]. These species signified that Okpoka Creek is under stress or suggested organic pollution at both tides from anthropogenic inputs from the surrounding industries and waterfront dwellers. Also, the presence of these species might further highlight inefficient disposal of domestic wastes in this creek. *Nitzschia lanceolata*, *N. filiforms*, *N. longissima*, *N. spp.*, *Navicula amphibola*, and *N. spp.* were present only at high tide while *Tabellaria fenestrate*, *Cymbella cuspidata*, *Pinnularia major*, *N. paradoxa*, *Navicula gracilis*, *N. cuspidata*, *N. placentula*, and *N. microcephala* were significantly abundant at high tide. *Cyclotella meneghiniana* was not significantly abundant at both tides but higher at high tide. This showed that these species exhibit migratory behaviour which is aided by passive transport. *Cocconeis placentula* was present only at low tide while *Synedra ulna*, *Nitzschia bilobata,* and *Navicula bacillum* were significantly abundant at low tide. *Nitzschia sigma* was in significantly abundant at both tides but higher at low tide. Advection cycle, settling, and resuspension that follow the suspended particles during the ebb, slack, and flood tidal stages may explain this observation. These species could be autochthonous phytoplankton so they were able to remain and possibly grew at a very high rate at low tide. Autochthonous

phytoplankton population has the ability to maintain itself within an estuarine segment and is determined mainly by its rate of growth relative to the proportion of population biomass lost through flushing from the segment during each tidal cycle [4]. Phytoplankton organisms may also use the capacity to grow at very high rates as a physiological response in order to compensate for advective losses during the ebb tide. Diatoms have the capacity for rapid cell division with growth rates (up to 5.9 day^{-1}). The flushing action of the tidal flows contributed to movement of pollutants (discharged into estuary by industries concentrated along it) down into the Okpoka Creek. Tide had significant effect on similarity index (P<0.05, DMR), higher at low tide (70.58±2.20%) than at high tide (59.35± 4.10%). This showed that diatoms were similarly distributed at low tide (Table 4).

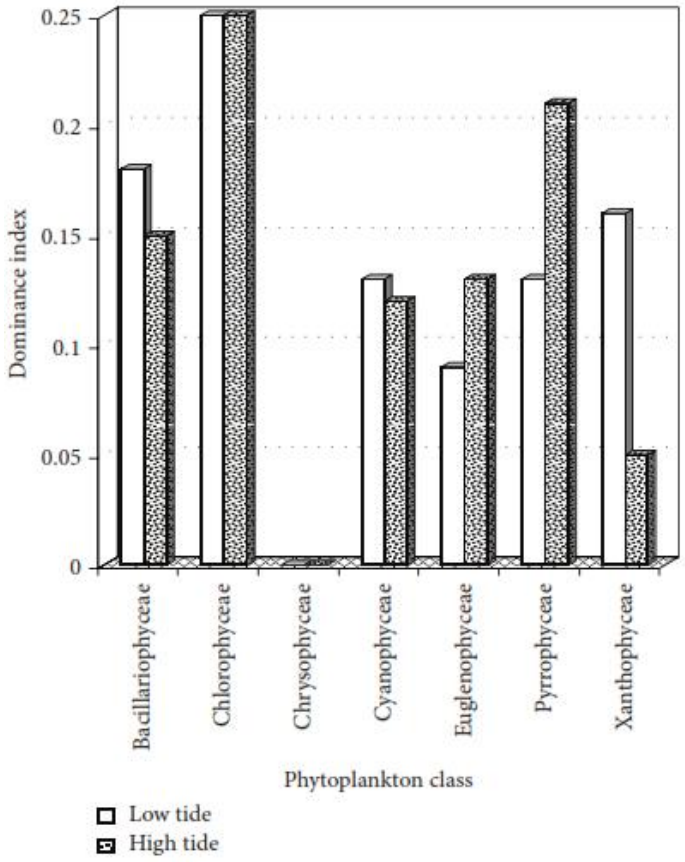


Fig. 7. Variation of phytoplankton dominance index in relation to tide in Okpoka Creek

4.4 Chlorophyceae (Green Algae)

Green algae density ranged between 1655.08 ± 126.62 no/mL (low tide) and 1729.59±204.6 no/mL (high tide) with a mean of 1672.97±107.89no/mL which was 14.68% total phytoplankton abundance. Tide had significant impact on parameters except density and Dominance index (P<0.05). The insignificant tidal differences in the density and dominance species index might be that the passive drifting of green algae from the sea into the Okpoka Creek via the Upper Bonny Estuary was probably low. These further suggested that tide had little or no effect on green algae populations. Also, it might be that the green algae of the Upper Bonny Estuary are environment selective or low in abundance. Diversity indices varied between low and high tides as follows: Margalef (0.52 ± 0.50 and 0.67±0.51), Shannon (0.30±0.02 and 0.37±0.03), Evenness (0.27±0.01and 0.33±0.02), and Dominance (0.25 ± 0.01 and 0.25±0.02).

Nine genera and 10 species out of the 44 observed green algae species were abundant (Table 5). The species were as follows: *Coelochaeta nitellarum* (76.16±16.16%), *Netrium digitus* (44.19± 4.72%), *Draparnaldia* sp. (42.392±1.92%), *Entransia dichloroplastes* (23.85±16.16%), *Crucigenia fenestrata* (36.39±2.70%), *Bulbochaeta* sp. (20.48±12.86%), *Gonatozygon aculeatum* (29.69 ± 3.23%), *Microspora villena* (22.35 ± 2.33%), *C. rectangularis* (21.47±2.83%), and *Coelastrum microsporum* (20.04±9.14%). Tidal effects on these green algae were different (P<0.05). It was observed that *Coleochaete nitellarum*, *E. dichloroplastes, Gonatozygon aculeatum*, *Netrium digitus*, and *Bulbochaeta* sp. were present only at low tide while *C. fenestrata* and *Microspora villeana* were significantly present at both tides (P<0.05) but their abundances were higher at low tide. This probably signified that at high tide, these dominant green algae species might rarely drif t into the creek thus the higher percentage distributions at low tide. The variation of abundant of *Draparnaldia* sp., *C. rectangularis*, and *Coelastrum microsporum* were significantly higher at high tide than low tide. The presence of *Cladophora glomerata* (especially at low tide) and *Scenedesmus* species (only at low tide) confirms the polluted nature of Okpoka Creek and agrees with Nwankwo and Akinsoji [51] that *C. glomerata* is tolerant to organic pollution and weak salinity. Weak salinity is expected at low tide as there is no inflow of sea water into the Okpoka Creek via the Bonny Estuary. The mean species similarity index of 43.60±2.92% and dissimilarity index of 56.40 ± 2.90% were recorded. Green algae species were similarly distributed at both tides.

4.5 Cyanophyceae (Blue-Green Algae)

Cyanophytes ranged from 369.47±31.52 no/mL (low tide) to 418.18±53.31 no/mL (high tide) with a mean of 382.31±27.10 no/mL and represented 3.36% of the phytoplankton population.

Table 3. Variations of Bacillariophyceae species under tidal influence in Okpoka Creek

		Mean ± SEM (%)			
S/No	**Species**	**Overall**	**Low tide**	**High tide**	**Statistical difference (ANOVA)**
1	*Cyclotella comta*	10.89 ± 1.32	11.26 ± 1.54[a]	8.91 ± 1.22[b]	P<0.05
2	*C. meneghiniana*	9.34 ± 0.97	9.34 ± 1.14 [a]	9.36 ± 1.94 [a]	P>0.05
3	*C. striata*	7.31 ± 1.24	5.96 ± 1.36 [b]	8.65 ± 2.02 [a]	P>0.05
4	*C. operculata*	17.24 ± 1.63	17.03 ± 1.98[a]	18.33 ± 0.00 [a]	P>0.05
5	*C. species**	3.96 ± 0.32	3.96 ± 0.32	--------	
6	*Fragilaria intermedia*	1.94 ± 0.54	1.85 ± 0.32 [a]	2.12 ± 1.91 [a]	P>0.05
7	*F. capucina*	2.66 ± 1.59	2.66 ± 1.59	--------	
8	*F. construens*	0.39 ± 0.00	--------	0.39 ± 0.00	
9	*F. species**	3.02 ± 0.00	--------	3.02 ± 0.00	
10	*Epithermia zebra*	0.47 ± 0.00	0.47 ± 0.00	--------	
11	*Attheya zacharias*	0.79 ± 0.00	--------	0.79 ± 0.00	
12	*Stauroneis acuta*	3.21 ± 0.00	3.21 ± 0.00	----------	
13	*S. parvula*	2.71 ± 0.00	----------	2.71 ± 0.00	
14	*S. species**	1.97 ± 0.00	---------	4.36 ± 1.03	
15	*Diatoma vulgare*	4.35 ± 0.77	4.33 ± 1.21[a]	0.65 ± 0.36[b]	P<0.05
16	*Rhizosolenia species**	0.65 ± 0.36	---------	1.69 ± 0.34	
17	*Tabellaria fenestrate*	1.62 ± 0.26	1.34 ± 0.02[b]	12.81 ± 1.84[a]	P<0.05
18	*Synedria ulna*	12.08 ± 1.71	8.80 ± 4.64[a]	3.82 ± 0.00[b]	P<0.05
19	*S. acus*	4.51 ± 0.69	5.19 ± 0.00[b]	8.33 ± 1.41[a]	P<0.05
20	*S. affinis*	7.29 ± 1.55	2.12 ± 0.00 [b]	8.81 ± 1.27[a]	P<0.05
21	*S. species**	10.26 ± 1.26	14.14 ± 1.96[a]	9.90 ± 1.22[b]	P<0.05
22	*Coslnodiscus lacustris*	9.46 ± 1.00	7.81 ± 1.52 [a]	5.64 ± 1.55[b]	P<0.05
23	*C. radiata*	6.39 ± 1.18	7.90 ± 1.71[a]	0.96 ± 0.00[b]	P<0.05
24	*C. excentricus*	0.96 ± 0.00	---------	7.41 ± 6.61	
25	*C. species**	7.41 ± 6.61	---------	2.53 ± 0.66	
26	*Amphora ovalis*	2.68 ± 0.54	3.17 ± 0.86[b]	15.48 ± 1.76[a]	P<0.05
27	*Nitzchia sigma*	15.15 ± 1.64	11.84 ± 4.46 [a]	10.16 ± 2.78 [a]	P>0.05
28	*N. bilobata*	11.39 ± 1.95	13.86 ± 1.55 [a]	8.50 ± 2.45 [b]	P<0.05

		Mean ± SEM (%)			
S/No	**Species**	**Overall**	**Low tide**	**High tide**	**Statistical difference (ANOVA)**
29	*N. lanceolata*	8.50 ± 2.45	---------	6.64 ± 0.70	
30	*N. paradoxa*	6.83 ± 0.60	7.75 ± 0.00 [b]	11.33 ± 0.85 [a]	P<0.05
31	*N. filiforms*	11.33 ± 0.85	---------	10.17 ± 0.00	
32	*N. longissima*	10.17 ± 0.00	---------	7.08 ± 3.47	
33	*N. species**	7.08 ± 3.47	---------	8.06 ± 1.60	
34	*Navicula gracilis*	7.15 ± 1.54	3.49 ± 0.00 [b]	11.29 ± 1.73 [a]	P<0.05
35	*N. cuspidata*	10.74 ± 1.60	6.91 ± 0.00 [b]	10.21 ± 0.71 [a]	P<0.05
36	*N. placentula*	10.09 ± 0.60	9.53 ± 1.07 [b]	12.41 ± 1.11 [a]	P<0.05
37	*N. microcephala*	10.33 ± 1.72	7.21 ± 3.09 [b]	10.18 ± 2.11 [a]	P<0.05
38	*N. bacillum*	11.37 ± 1.18	12.55 ± 1.06 [a]	0.37 ± 0.00 [b]	P<0.05
39	*N. amphibola*	0.37 ± 0.00	--------	9.53 ± 2.95	
40	*N. species**	9.53 ± 2.95	----------	13.14 ± 0.73	
41	*Melosira varians*	12.54 ± 0.73	10.13 ± 2.07 [b]	26.03 ± 16.05 [a]	P>0.05
42	*M. undulata*	26.03 ± 16.05	---------	17.66 ± 0.96	
43	*M. pusilla*	17.66 ± 0.96	---------	14.62 ± 5.73	
44	*M. distans*	12.16 ± 4.13	7.23 ± 0.00 [a]	7.85 ± 0.25 [a]	P>0.05
45	*M. listans*	9.08 ± 0.99	10.92 ± 2.01	--------	
46	*M. granulata*	1.60 ± 0.00	1.60 ± 0.00 [a]	0.77 ± 0.00 [a]	P>0.05
47	*M. species**	0.77 ± 0.00	----------	3.16 ± 0.01	
48	*Cymbella cuspidata*	2.34 ± 0.82	0.70 ± 0.00 [b]	2.20 ± 0.00 [a]	P<0.05
49	*C. lata*	2.20 ± 0.00	--------	3.59± 0.01	
50	*Aterionella formosa*	0.84 ± 0.00	0.84 ± 10.00[b]	2.41 ± 0.00 [a]	P<0.05
51	*Surirella species**	2.41 ± 0.00	--------	1.94 ± 0.00	
52	*S. tenera*	1.25 ± 0.70	0.55 ± 0.00	0.99 ± 0.00	
53	*S. elegans*	0.99 ± 0.00	--------	4.66 ± 2.25	
54	*S. robusta*	5.49 ± 2.08	10.48 ± 0.00 [a]	0.27 ± 0.00 [b]	P<0.05
55	*Cymtopleura sp*	0.27 ± 0.00	---------	1.16 ± 0.00	
56	*Campylodiscus hibernicus*	1.16 ± 0.00	---------	2.30 ± 1.18	
57	*Pinnularia viridis*	2.03 ± 0.73	---------	1.75 ± 0.30	
58	*P. horealis*	2.90 ± 0.82	4.05 ± 1.39[a]	0.56 ± 0.21[b]	P<0.05
59	*P. major*	0.87 ± 0.33	1.49 ± 0.00[b]	6.69 ± 1.10 [a]	P<0.05
60	*P. species**	6.69 ± 1.10	---------	7.00 ± 1.12	
61	*Frustulia rhomboides*	7.35 ± 0.96	8.40 ± 2.04[a]	7.08 ± 0.81[a]	P>0.05
62	*Gyrosigma acuminatum*	7.24 ± 0.75	7.76 ± 1.88[b]	15.76 ± 0.00 [a]	P<0.05
63	*G. species**	15.76 ± 0.00	----------	11.46 ± 0.00	
64	*G. attenuatum*	11.46 ± 0.00	--------	3.53 ± 0.00	
65	*G. paradoxa*	3.53 ± 0.00	---------	5.61 ± 1.08	
66	*Stephanodiscus astrae*	6.10 ± 1.07	9.98 ± 0.00	--------	
67	*S. species**	1.70 ± 0.00	1.70 ± 0.00	--------	
68	*Cocconeis placetula*	0.38 ± 0.00	0.38 ± 0.00	-------	
69	*Bacteriastrum*	0.10 ± 0.00	0.10 ± 0.00	--------	
70	*Bacillaria species**	1.57 ± 0.00	1.52 ± 0.00	---------	
71	*Biddulphia species**	0.30 ± 0.00	0.30 ± 0.00	--------	
72	*Corethron hystrix*	10.82±0.00	10.82 ± 0.00	--------	
73	*Cylindrotheca species**	4.67 ± 0.00	4.67 ± 0.00	---------	
74	*C. gracillis*	2.67 ± 0.00	2.67 ± 0.00	---------	
75	*Diploneis species**	0.07 ± 0.00	0.07 ± 0.00	--------	
76	*Ditylum species**	0.10 ± 0.00	0.10 ± 0.00	--------	
77	*Hydrosera species**	1.00 ± 0.00	1.00 ± 0.00	---------	
78	*Skeletonema species**	0.84 ± 0.00	0.84 ± 0.00	---------	
79	*Thalossiothrix species**	0.13 ± 0.00	0.13 ± 0.00	---------	
80	*T. longissimum*	0.17 ± 0.00	0.17 ± 0.00	--------	

*Means with the same letter in the same row are not significantly different (P > 0.05). *Means unidentified; —: absent.*

Table 4. Variations of phytoplankton similarity and dissimilarity indices in relation to tide in Okpoka Creek

				Similarity Index (%)			
Tide	**Bacillariophyceae**	**Chlorophyceae**	**Cyanophyceae**	**Xantophyceae**	**Pyrrophyceae**	**Chrysophyceae**	**Euglenophyceae**
Low	70.58±2.20[a]	43.75±3.40[a]	17.38±3.44[a]	38.68±5.57[a]	12.32±4.85[b]	7.24±7.14[a]	9.82±4.19[a]
High	59.35±4.10[b]	43.08±5.61[a]	21.71±6.94[a]	13.40±7.16[a]	40.00±24.50[a]	00.00±0.00[a]	9.57±6.50[a]
				Dissimilarity Index (%)			
Low	29.42±2.15[b]	56.25±3.39[a]	82.62±3.43[a]	61.32±5.62[a]	87.68±6.86[a]	82.76±7.14[a]	90.18±6.87[a]
High	40.65±4.10[a]	56.92±5.61[a]	78.29±6.94[a]	86.60±7.16[a]	60.00±24.50[a]	100.00±0.00[a]	90.43±5.58[a]

Means with the same letter in the same column are not significantly different (P>0.05)

Table 5. Variations of Chlorophyceae species under tidal influence in Okpoka Creek

S/No.	Species	Overall	Mean ± SEM (%)		
			Low tide	High tide	Statistical difference (ANOVA)
1	*Microspora villeana*	22.35 ± 2.33	23.15 ± 2.89[a]	19.18 ± 0.61[b]	P<0.05
2	*Spirotaenia species**	11.88 ± 4.12	11.88 ± 4.12	---------	
3	*Coelochaeta nitellarum*	76.16 ± 16.16	76.16 ± 16.16	---------	
4	*Mesotaenium species**	16.08 ± 8.17	26.55 ± 13.46[a]	5.59 ± 0.24[b]	P<0.05
5	*Entransia dichloroplastes*	23.85 ± 16.16	23.85 ± 16.16	----------	
6	*Chactosphora species**	1.47 ± 0.00	1.47 ± 0.00	----------	
7	*Tetraedron tumidulum*	7.53 ± 2.51	8.48 ± 3.04[a]	3.76 ± 2.45[b]	P<0.05
8	*Tetmemorus species**	1.77 ± 0.00	----------	1.77 ± 0.00	
9	*Penium species**	2.07 ± 0.00	2.07 ± 0.00	----------	
10	*Crucigenia rectangularis*	21.47 ± 2.83	18.13 ± 2.03[b]	28.15 ± 6.81[a]	P<0.05
11	*C. fenestrata*	36.39 ± 2.70	37.62 ± 2.73[a]	26.56 ± 0.00[b]	P<0.05
12	*C. puadrata*	13.65 ± 3.62	12.66 ± 4.92[b]	16.61 ± 0.00[a]	P<0.05
13	*Gonatozygon aculeatum*	29.69 ± 3.23	29.69 ± 3.23	----------	
14	*G. species**	12.18 ± 6.39	12.18 ± 6.39	----------	
15	*Staurastrum grande*	5.90 ± 2.21	7.36 ± 2.88[a]	2.99 ±0.00[b]	P<0.05
16	*S. natator*	14.29 ± 0.00	14.29 ± 0.00[a]	6.18 ± 0.02[b]	P<0.05
17	*Closterium dianae*	6.18 ± 0.02	----------	21.59 ± 0.92	
18	*C. parvulum*	16.16 ± 2.49	10.74 ± 2.92[a]	7.21 ± 2.12[b]	P<0.05
19	*C. gracile*	7.81 ± 1.75	10.19 ± 2.11[a]	2.36 ± 0.00[b]	P<0.05
20	*C. kuetzingii*	2.36 ± 0.00	--------	1.81 ± 0.00	
21	*C. macilentum*	1.81 ± 0.00	--------	4.72 ± 0.00	
22	*C. strigosum*	4.72 ± 0.00	---------	5.01± 0.01	
23	*Cosmarium species**	7.14 ± 0.00	7.14 ± 0.00[b]	20.00 ± 0.00[a]	P<0.05
24	*C. granatum*	11.40 ± 8.60	2.80 ± 0.00[b]	13.42 ± 0.00[a]	P<0.05
25	*Cladophora glomerata*	15.94 ± 2.52	18.45 ± 0.00[a]	9.73 ± 5.55[b]	P<0.05
26	*C. elegans*	12.21 ± 2.98	14.69 ± 2.71[b]	64.99 ± 30.82[a]	P<0.05
27	*Draparnaldia species**	42.39 ± 21.92	8.50 ± 6.47[b]	24.89 ± 18.58[a]	P<0.05
28	*Celatrium micromium*	20.04 ± 9.14	15.80 ± 10.95[b]	18.96 ± 3.96[a]	P<0.05
29	*C. recticulatum*	16.62 ± 3.28	8.82 ± 2.44[b]	13.33 ± 0.00[a]	P<0.05
30	*Rhizodonium hookeri*	12.98 ± 0.36	12.62 ± 0.00[a]	1.01 ± 0.00[b]	P<0.05
31	*Desmidium grevillii*	1.52 ± 0.51	2.03 ± 0.00[b]	11.53 ± 2.29[a]	P<0.05
32	*D. aptogonum*	8.62 ± 3.19	2.80 ±0.00[b]	5.09 ± 0.00[a]	P<0.05
33	*Planktosphueria gelatinosa*	5.21 ± 0.12	5.33 ± 0.00[b]	43.71 ± 5.06[a]	P<0.05
34	*Netrium digitus*	44.19 ± 4.72	1.72 ± 0.00	----------	
35	*Treubaria crassispina*	1.72±0.00	20.48 ± 12.86	----------	
36	*Bulbochaeta species**	20.48±12.86	12.90 ± 0.44	----------	
37	*Closteridium lunula*	12.90 ± 0.44	7.62 ± 0.00	----------	
38	*Docidium species**	7.62 ± 0.00	44.44±0.00	----------	
39	*Ankistrodesmus species**	44.44 ± 0.00	7.41±0.00	----------	
40	*Oedogonium species**	7.41 ± 0.00	38.33 ± 0.00	----------	
41	*Scenedesmus species**	38.33 ± 0.00	14.82 ± 0.00	----------	
42	*Spirogyra species**	14.82 ± 0.00	2.07 ± 0.00	----------	
43	*Cladophora species**	2.07 ± 0.00	17.15 ± 2.86	----------	
44	*Netrium species**	17.15 ± 2.86	18.05± 3.05	----------	

Means with the same letter in the same row are not significantly different (P > 0.05).
**Means unidentified; —: absent.*

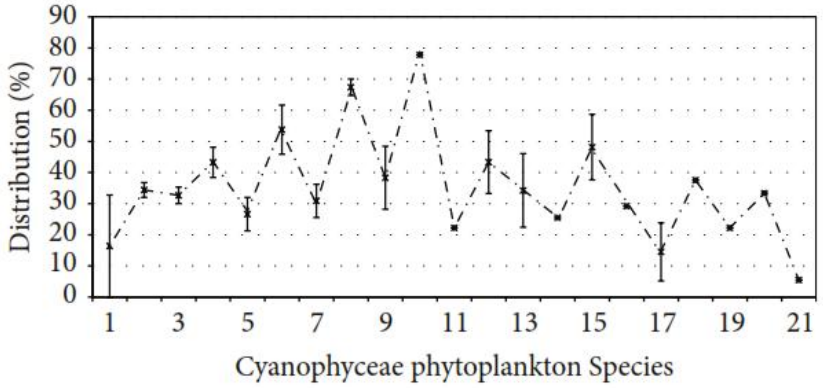


Fig. 8. Overall mean values of Cyanophyceae species in Okpoka Creek. (1 = *A. sp*, 2 = *A. affinis*, 3 = *A. arnoldii*, 4 = *A. spiroides*, 5 = *A. arnoldii*, 6 = *S. subsalsa*, 7 = *S. laxissima*, 8 = *S. major*, 9 = *S. princeps*, 10 = *M. pulverea*, 11 = *O. princeps*, 12 = *O. lacustris*, 13 = *O. tenuis*, 14 = *O. sp*, 15 = *R. sp*, 16 = *L. hieronymussi*, 17 = *L. limnetica*, 18 = *L. major*, 19 = *R. curvata*, 20 = *P. musicola*, and 21 = *N. sp*)

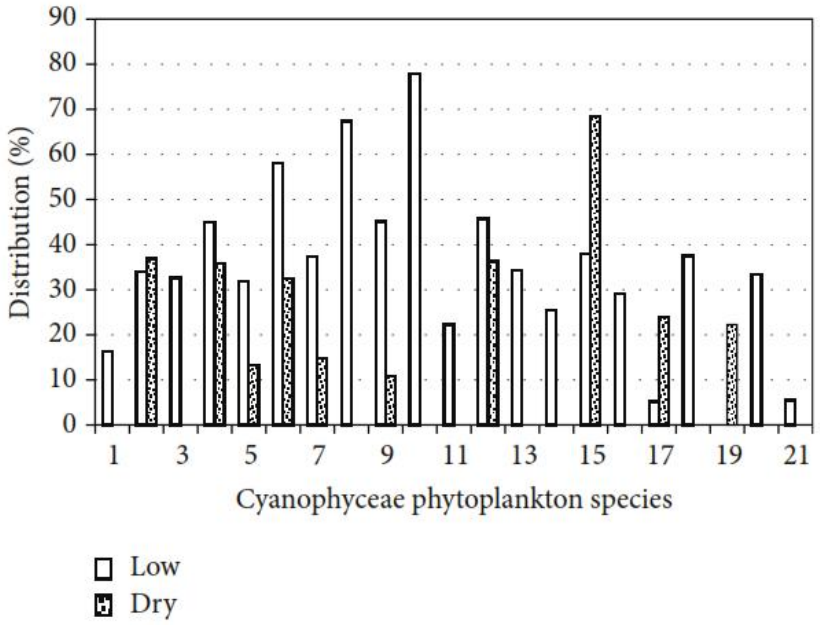


Fig. 9. Variation of Cyanophyceae species in relation to tide in Okpoka Creek. (1 = *A. sp*, 2 = *A. affinis*, 3 = *A. arnoldii*, 4 = *A. spiroides*, 5 = *A. arnoldii*, 6 = *S. subsalsa*, 7 = *S. laxissima*, 8 = *S. major*, 9 = *S. princeps*, 10 = *M. pulverea*, 11 = *O. princeps*, 12 = *O. lacustris*, 13 = *O. tenuis*, 14 = *O. sp*, 15 = *R. sp*, 16 = *L. hieronymussi*, 17 = *L. limnetica*, 18 = *L. major*, 19 = *R. curvata*, 20 = *P. musicola*, and 21 = *N. sp*)

The ANOVA for these phytoplankton parameters showed insignificant difference (P>0.05). The insignificant higher density of blue-green algae at high tide might be traced to inflow of these plants from the Upper Bonny Estuary into this creek. The slightly higher Margalef species richness and dominance index at low water showed that green algae species might have the ability to sink and settle vertically down within the water column; thus, they were retained in Okpoka Creek at low tide. Also, this observation might indicate a stable green algae community at low tide. From Fig. 8, the dominant cyanophytes genera were *Spirulina*, *Rivularia*, *Oscillatoria*, and *Anabaena.* There were 10

salient species, namely; *Spirulina major* (67.39±2.65%), *S. subsalsa* (53.72±7.90%), *Rivularia sp* (48.12±10.51%), *Oscillatoria lacustris* (43.31±10.10%), *Anabaena spiroides, Spirulina princeps,* (38.27±10.11%), *O. tenuis* (34.21±11.82%), and *A. arnoldii* (32.65±2.65%). Tide had varied influence on these species. *A. arnoldii, S. major* and *O. tenuis* were only present at low tide. *S. affinis*, *O. lacustris*, and *Rivularia sp* were higher in distribution at high tide than at low tide while other species showed opposite response to tidal effect (Fig. 9). *Anabaena spiroides* and *Microcystis pulverea* are pollution indicator species and were more abundant at low tide. These species are associated with eutrophic water bodies [56] and might have occurred in Okpoka Creek due to the increased influx of nutrients from anthropogenic inputs. Their presence at both tides except *Microcystis pulverea* could suggest pollution at both tides. At high tide, pollutants from the Upper Bonny Estuary moved into the creek while at low tide, pollutants from the different industries and residential buildings were pronounced. The mean species similarity index (18.41±3.08%) and dissimilarity index of (81.59±33.49%) indicated different distributions of species at low and high tides. There was a higher insignificant dissimilarity index at low tide (82.62±3.43%) than at high tide (78.29±6.94%) (P>0.05).

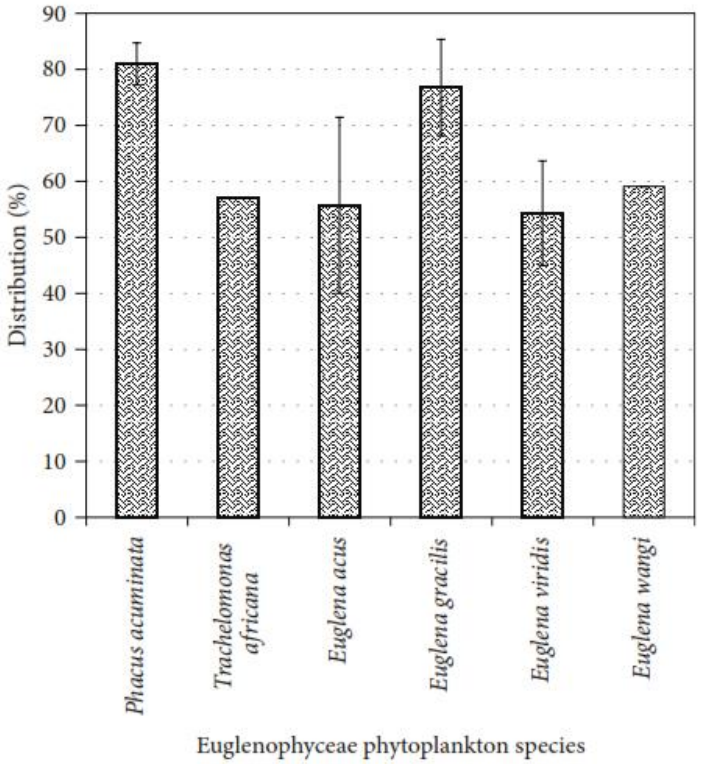


Fig. 10. Overall mean values of Euglenophyceae species in Okpoka Creek

4.6 Euglenophyceae (Euglenoid Flagellates)

Euglenophyceae accounted for 0.85% (the least) of the phytoplankton population. Insignificant higher density of algae (105.74±19.55 no/mL) was recorded at low tide than at high tide (68.63±17.78 no/mL) (P>0.05). Diversity indices were significantly higher at high tide than at low tide (P<0.05). Shannon index was insignificantly higher at low tide while evenness index was significantly higher at low tide (P<0.05, DMR). This indicated that euglenoid species diversity was higher and unevenly distributed at high tide. This might be attributed to tidal turbulence owning to the inflow of sea water into the creek. Three genera and 6 species, namely, *Phacus acuminata* (80.95±3.75%), *Euglena gracilis* (76.74±8.61%), *E. acus* (55.72±15.72%) *E. viridis* (54.32±9.32%), *E. wangi* (59.09±0.00%), and *Trachelomonas africana* (57.14±0.00%) were observed (Fig. 10). Tide had significant different effects on these species (P< 0.05) (Fig. 11).

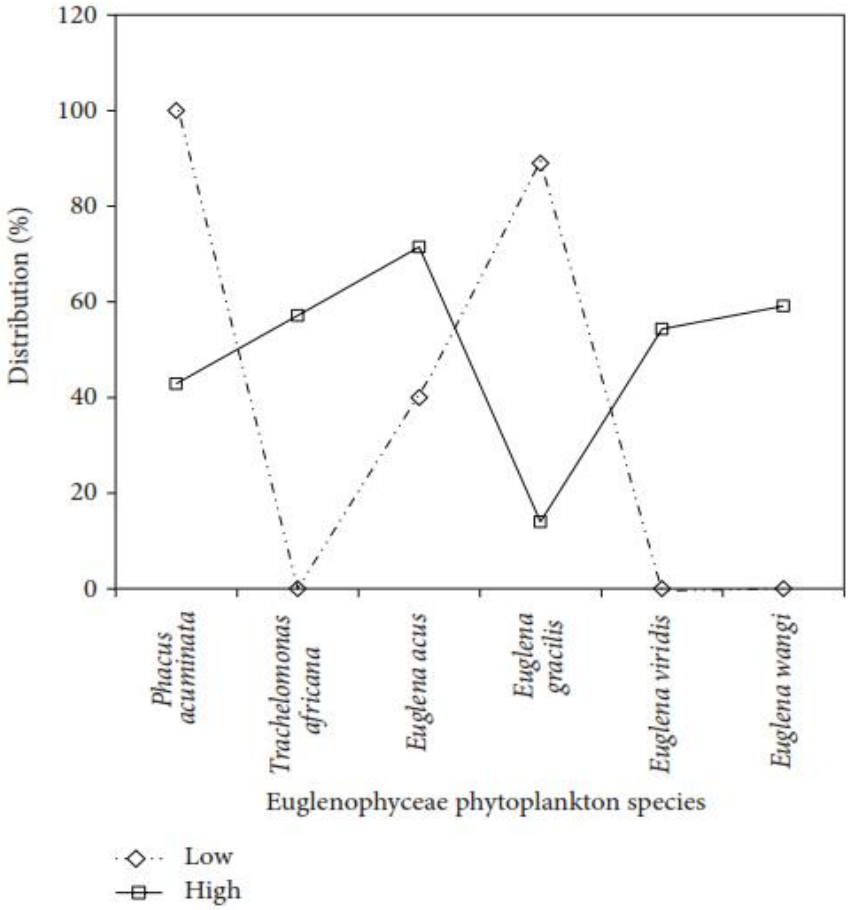


Fig. 11. Variations of Euglenophyceae species in relation to tide in Okpoka Creek

P. acuminata and *E. gracilis* were higher at low tide than at high tide. *T. africana*, *E. viridis* and *E. wangi* were absent at low tide. Only one species (*E. acus*) was higher at high tide (71.43±0.00%) than at low tide (40.00±0.00%). *E. acus*, *E. gracilis*, *E. viridis*, *E. wangi*, and *Phacus acuminata* are implicated to organic matter. Organic matter and iron are implicated to be among the ecological factors that affect the euglenoids [51]. Pollution by dissolved organic nutrients is best interpreted by the saprobic system. The presence of higher abundance of *E. acus* at high tide might be a pointer that Okpoka Creek is undergoing deterioration especially at high water. The influx of sea water from the sea via the Bonny Estuary brings in effluents from the numerous industries and waterfront dwellers sited along the water course. The mean similarity index was 8.07±3.37% and dissimilarity index 91.93 ±4.59%. Tide had no significant effect on similarity and dissimilarity indices. Species composition of euglenoid flagellates was dissimilar between the low tide (90.18±6.87%) and high tide (90.43±5.58%).

4.7 Pyrrophyceae (Dinoflagellates)

Dinoflagellates accounted for 2.52% of the phytoplankton abundance and ranged between 277.00± 65.80 no/mL (low tide) and 314.57±117.99 no/mL (high tide) with a mean of 286.74±56.46no/mL. Tidal influence on dinoflagellates parameters was insignificant (P>0.05). Other parameters ranged between low tide and high tide as follows: Margalef (0.19±0.09 and 0.26±0.13), Shannon (0.08±0.03 and 0.13±0.06), Evenness (0.12±0.05 and 0.19±0.09), and Dominance (0.13±0.05 and 0.21±0.10). Two genera and 4 species of dinoflagellates were observed (Fig. 12). They were *Ceratium furca*

(100.00±0.00%), *C. hirudinella* (81.42±1.11%), *Peridinium cinctum* (49.73±12.70%), and *P. umbonatum* (100.00±0.00%). Fig. 13 shows that *C. furca* and *P. umbonatum* were present only at low tide. The absence of these species at high tide might probably be adduced to tidally phototactic vertical migration pattern and endogenous tidal rhythm [4]. Before high tide, these organisms disappeared, swimming down and attaching themselves to the dark underside of solid objects so as not to be affected by the high water or rise in water level. *C. hirudinella* distribution percentage was high at high tide (80.44±19.57%) than at low tide (81.75±8.29%). *P. cinctum* tidal mean ranged between 39.13±0.00% (high tide) and 52.38±16.03% (low tide). These species that were present at both tides could indicate resilience of these algae. The observed higher abundances of dinoflagellates at low tide could suggest passive transport process, and it is the main factor controlling the permanence of these species. In addition, the ability these algae to grow at a faster rate might be the possible reason. Dinoflagellates have the capacity for rapid cell division with growth rates up to 2.7 day^{-1} based on equivalent body mass [4]. *Ceratium furca* has been implicated to organic pollution.

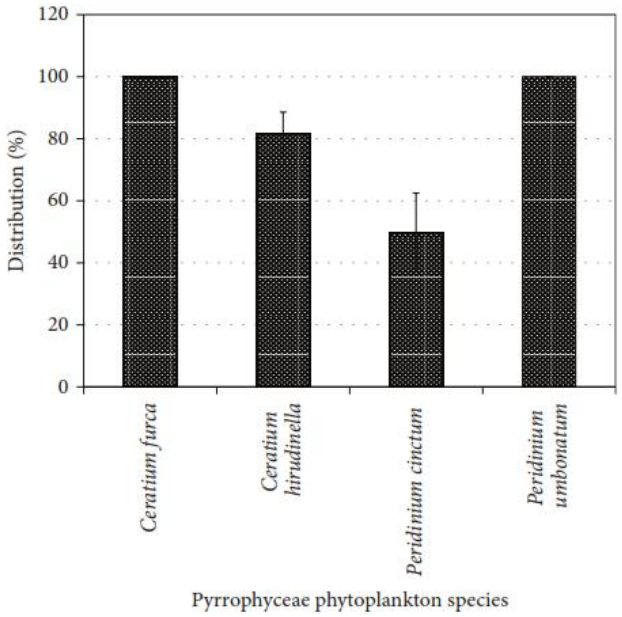


Fig. 12. Overall mean values of Pyrrophyceae species in Okpoka Creek

The mean similarity index ranged from 0.00±0.00% to 39.00±18.12% with a mean of 18.08±6.64%, and dissimilarity index ranged from 61.00±18.12 to 100.00±0.00% with a mean of 81.92±7.51%. The ANOVA showed no significant differences. Tide had no significant effect on similarity and dissimilarity indices of dinoflagellates. Similarity index ranged between 12.32±4.85% (low tide) and 40.00±24.50% (high tide) while dissimilarity index was 60.00±24.50% (high tide) and 87.68±6.86% (low tide).

4.8 Xanthophyceae (Xanthophytes)

Xanthophytes density ranged significantly between 1097.03±103.82 no/mL (low tide) and 2372.64± 243.52 no/mL (high tide) ($P<0.05$, DMR). The mean diversity indices were significantly high (DMR) at low tide than at high tide as follows: Margalef 0.03±0.02 and 0.14 ± 0.02, Shannon 0.04±0.02and 0.09±0.01, Evenness 0.04±0.03 and 0.13±0.02, and Dominance 0.05 ± 0.16 and 0.16 ± 0.02. Passive transport process, advection cycle, settling, and resuspension that follow the suspended particles during the ebb, slack, and flood tidal stages could be the possible reasons for this observation. Low tide similarity and dissimilarity indices were 38.68 ± 5.57% and 61.32±5.62%, respectively, while high tide similarity and dissimilarity indices were 13.40±7.16% and 86.60±7.16%, respectively. Tidal influences on these indices were not significant ($P>0.05$, DMR). This indicated that xanthophytes were not similarly distributed at low tide.

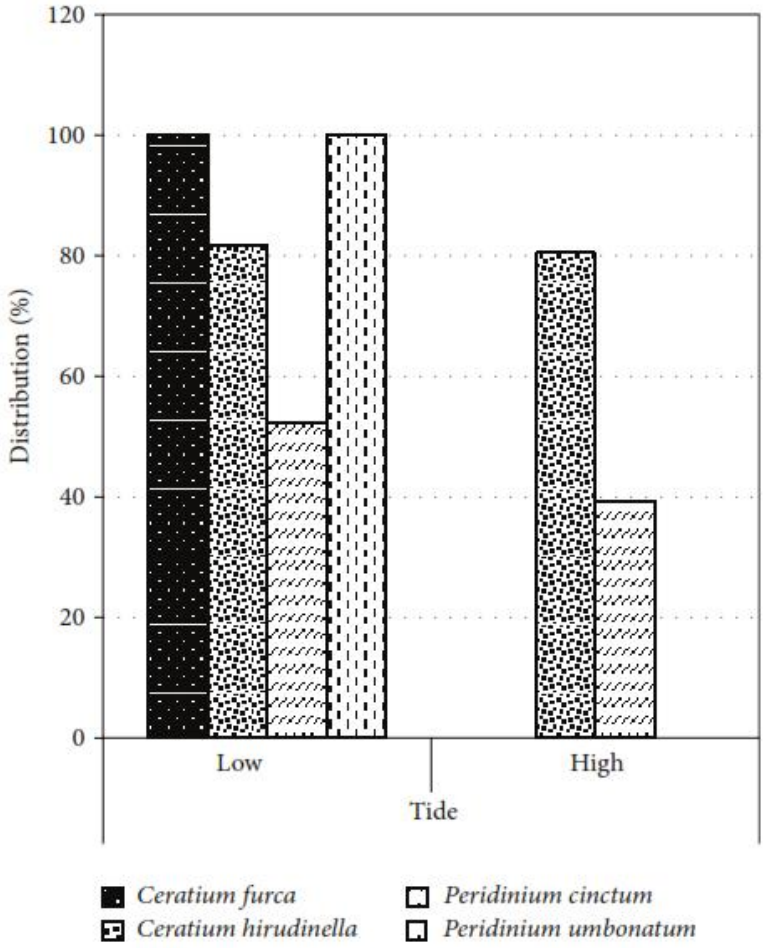


Fig. 13. Variation of Pyrrophyceae species in relation to tide in Okpoka Creek

One genus and 2 species of xanthophyceae, namely, *Tribonema viride* (53.21±10.58%) and *T. vulgare* (81.55±6.33%) were observed (Fig. 14). From Fig. 15, high tide distribution percentages were significantly higher than that of low tide percentages of abundance of species ($P< 0.05$, DMR). *T. viride* had distribution percentages of 50.29±11.39% at low tide and 64.90±35.11% at high tide. *T. vulgare* had distribution percentages of 79.85±7.37% at low tide and 90.07±9.93% at high tide.

4.9 Chrysophyceae (Chrysophytes)

Chrysophytes ranged insignificantly ($P>0.05$, DMR) from 124.25±33.99 no/mL (high tide) to 495.31±79.33 no/mL (low tide) with a mean of 457.267±3.46 no/mL which was 4.01% total phytoplankton abundance. The possible reasons for this observation are similar to those of xanthophytes. Also, it could indicate the stress condition of Okpoka Creek and that they are not resilient algae. All species diversity indices were zero signifying very low species diversity. Only 1 species, *Dinobryon sertularia,* was observed. This might mean similarity index (15.15±6.34%) and dissimilarity index (84.85±6.34%) of chrysophytes were recorded. Tide had no significant effects on similarity and dissimilarity indices ($P>0.05$, DMR). Dissimilarity index of species was higher at high tide indicating that chrysophytes were differently distributed at high tide.

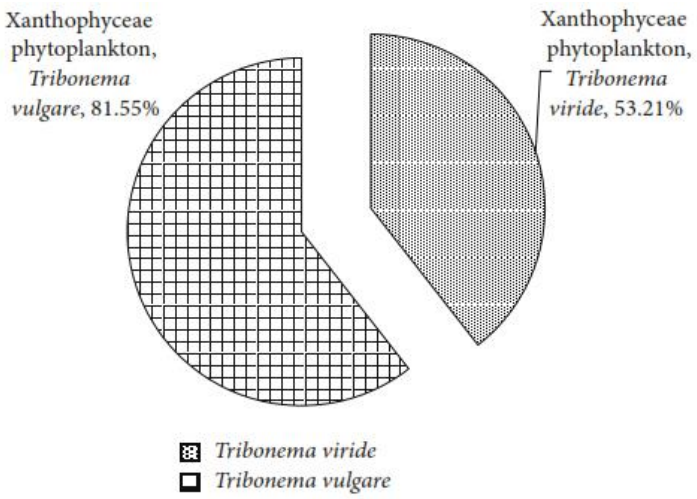


Fig. 14. Overall mean values of Xanthophyceae species in Okpoka Creek

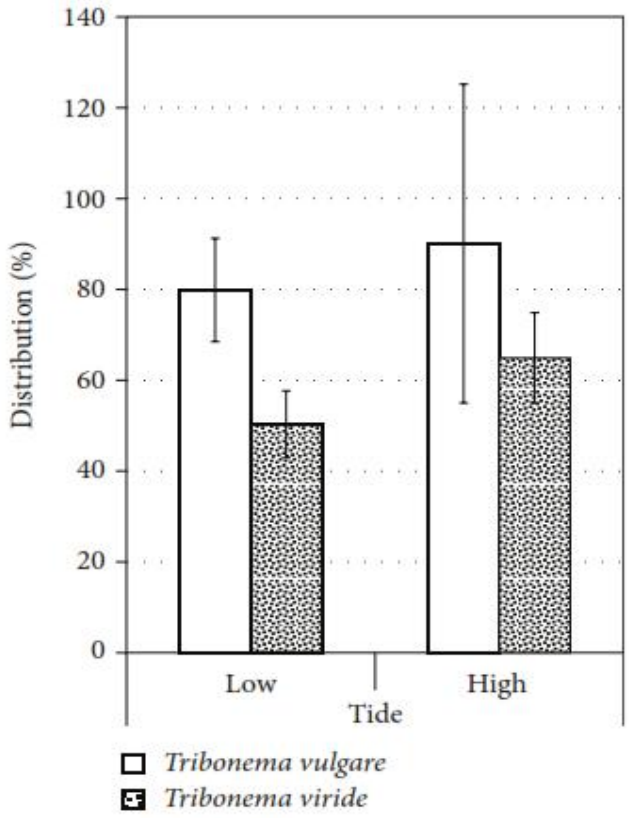


Fig. 15. Variation of Xanthophyceae species in relation to tide in Okpoka Creek

5. CONCLUSION

Tide has varied effects on the nutrients status and phyto-plankton community (in terms of species composition, diversity, abundance, and distribution) of Okpoka Creek. Phosphate and ammonia (organic pollutants) exceeded FEPA and USEPA acceptable levels of 0.10 mg/L and 0.10 mg/L,

respectively, for natural water bodies indicating high nutrient status, organic matter, and potential pollutants in this creek. Diatoms are the dominant phytoplankton while euglenoids are the passive (least) phytoplankters. They are sensitive and resilient to both low and high tides. Organic pollution indicator species: *Navicula gracilis, N. cuspidata, N. placentula, N. microcephala, N. bacillum, N. amphibola, Nitzschia sigma, N. bilobata, N. lanceolata, N. filiforms, N. longissima, Cymbella cuspidata, C. lata, Tabellaria fenestrate*, *Synedra ulna*, *Cyclotella meneghiniana*, *Cocconeis placentula*, *Pinnularia major* (diatoms), *Cladophora glomerata*, *Scenedesmus spp*, (green algae), *Euglena acus* (euglenoid), *Anabaena spiroides*, *Microcystis pulverea* (blue-green algae), and *Ceratium furca* (dinoflagellate) were recorded at either only low, high or both tides. The flushing action of the tidal flows contributes to moving these pollutants down into this creek.

6. RECOMMENDATIONS

Tide is a major determinant of tidal variations. This study therefore suggests the high-resolution monitoring programs that are essential to capture the natural variability of phytoplankton in coastal waters. Concerted environmental surveillance on Upper Bonny Estuary is advocated to reduce the inflow of pollutants from the Bonny Estuary into this creek caused by tidal influence. Aquatic scientists should be encouraged to conduct further researches on tidal effects on other aspects of the biology and ecology of phytoplankton in Okpoka Creek and other tributaries of Bonny Estuary as well as the Bonny Estuary at large in order to bridge the gap in knowledge of the abiotic and biotic properties of this estuary.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Badsi H, Oulad Ali H, Loudiki M, Aamiri A. Phyto-plankton diversity and community composition along the salinity gradient of the massa estuary. American Journal of Human Ecology. 2012; 1(2):58–64.
2. Wilcox BH. Angiosperm flora of the Niger Delta mangal: A taxonomic review. in Proceedings of Workshop on the Niger Delta Mangrove Ecosystem. Port Harcourt, Nigeria. 1980;19–23.
3. Villate F. Tidal influence on zonation and occurrence of resident and temporary zooplankton in a shallow system (Estuary of Mundaka, Bay of Biscay). Scientia Marina. 1997;61(2):173–188.
4. Trigueros JM, Orive E. Tidally driven distribution of phytoplankton blooms in a shallow, macrotidal estuary. Journal of Plankton Research. 2000;22(5):969–986.
5. Riaux-Gobin C. Phytoplankton, tripton et microphytobenthos: echanges´ au cours de la maree,´ dans un estuaire du Nord-Finistere.` Cahiers de Biologie Marine. 1987;28:159–184.
6. Morales-Zamorano LA, Cajal-Medrano R, Orellana-Cepeda E, Jimenez-Perez LC. Effect of tidal dynamics on a planktonic community in a coastal lagoon of Baja California, Mexico. Marine Ecology Progress Series. 1991;78(3):229–239.
7. Cloern JE. Tidal stirring and phytoplankton bloom dynamics in an estuary. Journal of Marine Research. 1991;49(1):203–221.
8. Lucas LV, Cloern JE. Effects of tidal shallowing and deepening on phytoplankton production dynamics: a modeling study. Estuaries A. 2002;25(4):497–507.
9. Blauw JAN, Beninca E,` Laane RWPM, Greenwood N, Huisman. Dancing with the tides: Fluctuations of coastal phytoplankton orchestrated by different oscillatory modes of the tidal cycle. PLoS ONE. 2012;7(11):Article ID e49319.
10. Rajesh KM, Gowda G, Mridula RM. Primary productivity of the brackishwater impoundments along Nethravathi estuary, Mangalore in relation to some physico-chemical parameters. Fishery Technology. 2002;39(2):85–87.
11. Ananthan G, Sampathkumar P, Soundarapandian P, Kannan L. Observations on environmental characteristics of Ariyankuppam estuary and Verampattinam coast of Pondicherry. Journal of Aquatic Biology. 2004;19:67–72.

12. Tiwari A, Chauhan SVS. Seasonal phytoplanktonic diversity of Kitham lake, Agra," Journal of Environmental Biology. 2006;27(1):35–38.
13. Tas B, Gonulol A. An ecologic and taxonomic study on phytoplankton of a shallow lake, Turkey. Journal of Environmental Biology. 2007;28(2):439–445.
14. Saravanakumar A, Sesh Serebiah J, Thivakaran GA, Rajkumar M. Benthic macrofaunal assemblage in the arid zone mangroves of gulf of Kachchh-Gujarat. Journal of Ocean University of China. 2007;6(3):303–309.
15. Ponmanickam P, Rajagopal, T Rajan MK, Achiraman S, Palanivelu K. Assessment of drinking water quality of Vembakottai reservoir, Virudhunagar district, Tamil Nadu. Journal of Experimental Zoology. 2007;10(2):485–488.
16. Shashi Shekhar TR, Kiran BR, Puttaiah ET, Shivaraj Y, Mahadevan KM. Phytoplankton as index of water quality with reference to industrial pollution. Journal of Environmental Biology. 2008;29(2):233–236.
17. American Public Health Association (APHA), Standard Method for the Examination of Water and WasteWater, McGraw-Hill, Washington DC, USA, 16th edition; 1985.
18. Boyd CE. Water quality in warmwater fish ponds. Craf tmaster, Auburn, Alabama, USA, 2nd edition; 1981.
19. Needham JG, Needham PR. A guide to the study of freshwater biology. Holden-Day, San Francisco, Calif, USA, 2nd edition; 1962.
20. Newell GE, Newell RC. Marine plankton: A practical guide, Hutchinson Publishing Limited, London, UK, 1st edition; 1963.
21. Patrick R, Reimer C. The diatoms of the United States exclusive Alaska and Hawaii T. Fragillariaceae, Eunoticeae, Achnanthaceae, Naviculaceae, Livingstone, Philadelphia, Pa, USA; 1966.
22. Han M. Illustration of freshwater plankton, Agricultural Press, Auburn, Alabama, USA, 1st edition, 1978. J. R. Durans and C. Leveque, "Flore et farune aquatiquesde 1, afrique-an-off erch," Science Technica Qutre-Mer. 1980;1:5– 46.
23. Prescott GW. How to know the freshwater algae. McGrawHill, Washington, DC, USA; 1982.
24. Kadiri MO. A taxonomic study of the genus Closterium (Nizch. 1919), Ralfs 1945 (Desmichaceae Chlorophyta) in small Nigeria Reservoir with ecological notes," Tropical Freshwater Biology. 1988;1:71–90.
25. Odum EP. Fundamentals of Ecology, W.B. Saunders, London, UK, 3rd edition; 1971.
26. Statistical Analysis System (SAS), User's Guide SAS/STA-T version, SAS Institute, Cary, NC, USA, 8th edition; 2003.
27. Yamamuro M, Koike I, Iizumi H. Partitioning of the nitrogen stock in the vicinity of a Fijian seagrass bed dominated by Syringodium isoetifolium (Ascherson) Dandy. Australian Journal of Marine & Freshwater Research. 1993;44(1):101–115.
28. Thurston RV, Russo RC, Vinogradov GA. Ammonia toxicity to fishes. Effect of pH on the toxicity of the unionized ammonia species. Environmental Science and Technology. 1981;15(7):837–840.
29. McNeely RN, Neimanis VP, Dwyer L. Water quality sourcebook: A guide to water quality parameters. Inland Waters Directorate, Water Quality Branch, Ottawa, Canada; 1979.
30. Environmental Studies Board (ESB). Water quality criteria 1972. Committee of Water Quality Criteria Report EPA-R3-73-033, Environmental Protection Agency, Washington, DC, USA; 1973.
31. Chindah AC, Nduaguibe U. Effect of tank farm wastewater on water quality and periphyton of Lower Bonny River Niger Delta, Nigeria. Journal of Nigeria Environmental Society. 2003;1(2): 206–222.
32. Obunwo CC, Braide SA, Izonfuo WAL, Chindah AC. Influence of urban activities on the water quality of a fresh water stream in the Niger Delta, Nigeria. Journal of Nigerian Environmental Society. 2004;2(2):196–209.
33. Zimmerman L. Phytoplankton-national estuarine research reserve; 2013. Available:http://nerrs.noaa.gov/doc/siteprofile/acebasin/ html/biores/phy
34. Frankovich TA, Gaiser EE, Zieman JC, Wachnicka AH. Spatial and temporal distributions of epiphytic diatoms growing on Thalassia testudinum Banks ex Konig:¨ relationships to water quality. Hydrobiologia. 2006;569(1):259–271.

35. Creswell J, Karasack R, Johnson R, Shayler H. Nitrogen and phosphorus in limitation in Mill and Green ponds and the effects of nutrient enrichment. Macalester Environmental Review; 2001.
36. Vitousek PM, Howarth RW. Nitrogen limitation on land and in the sea: How can it occur? Biogeochemistry. 1991;13(2):87–115.
37. European Economic Community (EEC). Council directive on the quality of freshwater needing protection or improvement in order to support fish life. Offshore Journal of European Commununities. 1979;L259:1–10.
38. United States Environmental Protection Agency (USEPA). Nitrate-Water; 2000. Available:http://water.epa.gov/type/rsl/monitoring/vms57.cfm.
39. United States Geological Survey (USGS). Nutrients in the Nation's Waters—Too Much of a Good Thing?; 2013.
Available:http:// pubs.usgs.gov/circ/circ1136/circ1136.html.
40. Ebere N. The impact of oil refinery effluents on the distribution, abundance and community structure of macrobenthos in Okrika Creek [Ph.D. thesis], Department of Biological Sciences, Rivers state University of Science and Technology; 2002.
41. Edoghotu AJ. The ecological quotients (EQ) of point source of pollution along Okpoka Creek, Port Harcourt [M.S. thesis], Department of Biological Sciences, Rivers State University of Science and Technology; 1998.
42. Chindah AC, Keremah RI. Physicochemistry and phytoplankton of a brackish water fish pond of the Bonny Estuary Nigeria. African Journal of Environmental Studies. 2001;2(2):63–67.
43. Chindah AC, Braide SA. Crude oil spill and the phytoplankton community of a swamp forest stream. African Journal of Environmental Studies. 2001;2(1):1–8.
44. Chindah AC, Braide SA. The physicochemical quality and phytoplankton community of tropical waters: A case of 4 Biotopes in the Lower Bonny River, Niger Delta, Nigeria. Caderno de Pesquisa. 2004;16(2):7–35.
45. Moser KA, MacDonald GM, Smol JP. Applications of freshwater diatoms to geographical research. Progress in Physical Geography. 1996;20(1):21–52.
46. Krumme U, Liang TH. Tidalinduced changes in a copepod-dominated zooplankton community in a macrotidal mangrove channel in northern Brazil," Zoological Studies. 2004;43(2):404–414.
47. Roelke DL, Errera RM, Riesling R, et al. Effects of nutrient enrichment on *Prymnesium parvum* population dynamics and toxicity: Results from field experiments, Lake Possum Kingdom, USA. Aquatic Microbial Ecology. 2007;46(2):125–140.
48. Passy SI, Bode RW, Carlson DM, Novak MA. Comparative environmental assessment in the studies of benthic diatom, macroinvertebrate, and fish communities. International Review of Hydrobiology. 2004;89(2):121–138.
49. Okpuruka DU. Tidal and semilunar variations in the surface phytoplankton of a Port Harcourt mangrove creek. Proceedings of the Workshop on Nigerian Wetlands, T. V. I. Akpata and D. U. U. Okali, Eds., pp. 1–183, Lagos, Nigeria; 1986.
50. Nwankwo DI, Akinsoji A. Periphyton algae of a eutrophic Creek and their possible use as indicator. Nigerian Journal of Botany. 1988;1:47–54.
51. Nwankwo DI, Onitiri AO. Periphyton community on submerged aquatic macrophytes (Hornwort and Bladderwort) in Epe Lagoon, Nigeria. Journal of Agricultural Science Technology. 1992; 2(2):135–141.
52. Nwankwo DI, Amuda SA. Periphytic diatoms on three floating aquatic macrophytes in a polluted south-west Nigerian creek. International Journal of Ecology & Environmental Sciences. 1993;19(1):1–10.
53. Nwankwo DI. Hydrochemical properties and bottom-dwelling diatoms of a Lagos lagoon sewage disposal site. Polskie Archiwum Hydrobiologii. 1994;41(1):35–47.
54. Nwankwo DI, Kasumulginla KA. Contributions to the diatom flora of Nigeria 11 Tubedwelling pinnate diatoms from Lagos Moles. Nigeria Journal of Botany. 1997;10:61–69.
55. Nwankwo DI. Seasonal changes in phytoplankton composition and diversity in the Epe lagoon, Nigeria. Acta Hydrobiology. 1998;40(2):83–92.

Biography of author(s)



Prof. O. A. Bubu-Davies
Department of Fisheries and Aquatic Environment, Rivers State University of Science and Technology, P.M.B. 5080, Port Harcourt, Nigeria.

She is a mentor and internationally visible, she is the first female Professor of Hydrobiology and Fisheries in the Department of Fisheries and Aquatic Environment, Rivers State University (RSU), Nigeria. She holds BSc Hons (Zoology), MSc and PhD in Zoology (Hydrobiology & Fisheries), all degrees from the premier University of Ibadan, Nigeria. She had her primary and secondary education at Abadina Primary School I and Onireke High School, Ibadan, Nigeria respectively. She is a Fellow of the Zoological Society of Nigeria, the prestigious African Women in Agricultural Research & Development (AWARD) Fellowship and the Netherlands Fellowship Programme (NFP). She won the highly competitive research attachment of AWARD and undertook a Post-Doc research and training on domestication of Black Tiger Shrimp, *Penaeus monodon* with Shrimp Genetic Improvement Centre, Phumriang, Chaiya, Surat Thani, Thailand, in 2011. Her life purpose is to fight poverty, hunger and unemployment in Nigeria and Africa by boosting fish production through sustainable aquaculture and capture fisheries. She has one hundred and ten (110) refereed journal articles (local & international) and five (5) conference proceedings papers and one (1) chapter in research book. She has presented 22 conference papers and 3 posters at local and international conferences. Some of her research articles are indexed in Scopus, Google Scholar, AJOL, ORCID, LiveDNA and Research Gate among other indexing bodies. Her areas of specialization are Zoology, Hydrobiology, Fisheries, Aquaculture, Aquatic Ecology/Pollution, Ecotourism, Biotechnology and Environmental Science. She has mentored many junior scientists, supervised many students (undergraduates and postgraduates) and served in many committees at RSU and Federal University, Otuoke (FUO), Nigeria. She is the immediate past Chairman, Senate Curriculum and Instructions Committee of RSU. Presently, she is a member of the University Curriculum Development Committee, FUO. She had held various administrative positions at RSU. She is the immediate past Editor-in-Chief, Journal of Aquatic Sciences, Nigeria (published by Association for Aquatic Sciences of Nigeria), and member of editorial board and peer reviewer of some reputable journals (local and international). She is a resource person to the National Commission of Universities on accreditation of academic programmes in Nigerian Universities, a fisheries/environmental consultant, external examiner, internal and external assessor to several universities in Nigeria. She is facilitator at different workshops on fish and snail farming organized by different organizations in Nigeria. However, she belongs to the following learned societies: Nigerian Women in Agricultural Research for Development 2013-date, World Aquaculture Society 2010-date, Fisheries Society of Nigeria 2005-date, Zoological Society of Nigeria 2002-date, Association for Aquatic Sciences of Nigeria 2012-date, American Society of Agricultural and Biological Engineers 2004-date, Multidisciplinary Aquatic Studies Team 2004-date.



Professor O. A. Ugwumba
Hydrobiology and Fisheries Unit, Department of Zoology, University of Ibadan, Ibadan 900001, Nigeria.

He has spent his early school years in Wilcox Primary School and Ngwa High School both in Aba, after which he proceeded to Baptist Academy, Shepherd Hill, Ikorodu Road Lagos for the higher School Certificate. He was then admitted into the University of Ibadan – 1976 – 1979 where he graduated with BSc. Honours in Zoology (2nd Class Upper Division). On completion of the National Youth Service in 1979/80, he proceeded to the University of Lagos where he obtained through joint partnership the Environmental Protection Agency Washington DC, a certificate in Waste management: EPA Cert. (1983) and bagged a PhD Degree in Zoology in 1984. In April 1985 he joined the staff of the Department of Zoology, University of Ibadan as Lecturer II. He rose to the rank of Professor of Zoology in 1999 and has mentored many students. He has over one hundred publications (journals, books and conference proceedings). He belongs to the following learned societies:Fellow, Nigeria Institute of Biology (FNIBIOL), Fellow Fisheries Society of Nigeria (Ffs), Fellow, Zoological Society of Nigeria(Fzsn), Member Association for Aquatic Sciences of Nigeria (AASN), Member Ecological Society of Nigeria, Member Network of Tropical Aquatic Scientists,

Member Network of Tropical Fisheries Scientists, Member Pan African Fish and Fisheries Association. He was Editor in Chief of The Zoologist 2001 – 2010, 1st National Vice President of Zoological Society of Nigeria 2011 – 2015 and presently he is the National President of Zoological Society of Nigeria 2015 to date. He has served in several committees, Boards and Halls of University of Ibadan at various times and he is External Examiner/Assessor in several Nigeria and West African Universities.

© Copyright (2021): Author(s). The licensee is the publisher (Book Publisher International).

DISCLAIMER
This chapter is an extended version of the article published by the same author(s) in the following journal.
Journal of Marine Biology, 2013: 16, 2013, Article ID 684739.

Chapter 9
Print ISBN: 978-93-90516-93-3, eBook ISBN: 978-93-90516-94-0

A Critical Study on Seasonal Variation in Major Minerals (Ca, P, K, Mg) and Proximate Composition in Flesh of Mesopotamian Catfish (*Silurus triostegus* Heckel, 1843) from Turkey

Mine Perçin Olgunoglu[1*], Ilkan Ali Olgunoğlu[1] and Mustafa Göçer[1]

DOI: 10.9734/bpi/rrab/v5/1703E

ABSTRACT

In this study, the seasonal variation on selected major minerals (Ca, P, K, Mg) and proximate composition of Mesopotamian catfish (*Silurus triostegus* Heckel, 1843) were investigated. The mineral and proximate composition of fish varies greatly from one species and one individual to another depending on age, sex, environment, season, area of catch and processing method. Fish samples used in this research were obtained seasonally from Atatürk Dam Lake within the boundries of Turkey (37ºC45N' latitude/38ºC17E' longitude) via fishing. The mean value of Ca, P, K and Mg were determined as 92.59±9.81 µg/g, 1447.56±117.38 µg/g, 2762.50±418.00 µg/g and 227.26±39.79 µg/g (wet weight) respectively. The highest mineral contents were identified in summer. The highest crude protein was observed in autumn (18.88%) and the lowest in winter (16.88%).The average lipid content was identified between 4.22-6.56% and the highest value was observed in winter. The results showed significant differences between the four seasons ($P<0.05$) in major minerals and proximate composition. The results of this study showed that Mesopotamian catfish from Atatürk Dam Lake (Turkey, Adiyaman) has rich lipid content, a low amount of major minerals and protein content when compared with other economical freshwater species from Turkey. However, the highest levels of the major minerals in Mesopotamian catfish (*S. triostegus*) were recorded in summer period.

Keywords: Mesopotamian catfish; Silurus triostegus; minerals; proximate composition.

1. INTRODUCTION

Fish protein and mineral contents are recognised for their nutritional and functional properties in human diet. There is growing evidence that fish lipids are good for the heart and blood vessels and control for cardiovascular diseases. Therefore eating fish or taking fish oil, both freshwater and marine, is being encouraged [1]. Strong links between fish and seafood consumption and positive health effects, especially with the decreased risk of coronary heart and cardiovascular diseases, decreased inflammatory disease as arthritis and prevention of cancer have been shown by many researchers [2-4]. There are two types of minerals: major minerals (Calcium, Phosphorus, Potassium, Magnesium etc.) which are essential to human health and play an important roles in biological systems required in higher amounts in the body and trace minerals (Iron, Zinc, Copper, Chromium etc), which amount to less than a teaspoon of a person's body weight [5-7]. The Information about some proximate parameters such as protein, lipids, carbohydrate, moisture and ash is often necessary to ensure that they meet the dietary requirements, food regulations and commercial specifications [8-11]. The mineral and proximate composition of fish varies greatly from one species and one individual to another depending on age, sex, environment, season, area of catch and processing method [7,12].

[1]*Department of Aquaculture and Fisheries Program, Kahta Vocational Training School, Adıyaman University, Turkey.*
**Corresponding author: E-mail: mineper@yahoo.com;*

Mesopotamian catfish (*S. triostegus*) is one of the leading fish species from the Atatü rk Dam Lake of Turkey (The Atatürk Dam Lake is one of the largest earth-and-rock filled dams in the world and has a high fishing potential) with great importance in economy and people who live in Southeastern Anatolia consume this fish abundantly [13-15]. There is no previous report on the major minerals and proximate composition of Mesopotamian catfish (*S. triostegus*). Therefore, the present study is aimed to see the seasonal variation in selected minerals and proximate composition in Mesopotamian catfish.

2. MATERIALS AND METHODS

2.1 Fish Samples

S. triostegus used in this research were obtained seasonally from Atatürk Dam Lake within the boundries of Turkey (37°C 45N' latitude/38°C 17 E' longitude) via fishing. Fish samples were stored in ice and transported to the laboratory on the same day. After removing the head, fins, scales, skin and all inner organs, fish muscle was washed with distilled water and placed in a polyethylene bag and kept at -20ºC until the analyses. A total of 40 (5 pairs of *S. triostegus* in each season) fish samples were used in the research.

2.2 Chemical Analysis

The flesh samples were transported with dry ice to the Industrial Services Laboratories of TUBITAK–MAM (The Scientific and Technological Research Council of Turkey, Marmara Reasearch Centre). The mineral analysis was carried out as described by Association of Official Analytical Chemists [16] using Atomic Absorption Spectrophotometer. 0.5g of fish muscle (wet weight) was weighed and placed in a teflon digestion vessel with 6ml of concentrated (65%) nitric acid (HNO_3) and 1.5ml 30% hydrogen peroxide (H_2O_2) and digested in microwave digestion system (Milestone Ethos PLUS). The major minerals determined were calcium (Ca), phosphorus (P), potassium (K) and magnesium (Mg). The concentrations were expressed as µg/g wet weight of tissue in organisms.

The protein analysis of flesh samples was carried out according to the Kjeldahl Method [17], and the fat estimation was done according to the Acid Hydrolosis Soxtec System [17], the moisture analysis was made by dehydrating the homogenized samples to a fixed weight with an incubator [17] and the raw ash was measured after burning the samples at 550ºC [17] The energy calculation of samples were evaluated with the Method of Watt and Merril [18].

2.3 Statistical Analysis

For data analysis, standard deviation and one-way ANOVA were employed by using SPSS 15.0 Windows software and Duncan's test was used in the evaluation between the means Significance of differences was defined at P≤0.05. The mean values were obtained from 3 experiments and reported as X±SD [19].

3. RESULTS AND DISCUSSION

The results of seasonal changes in major minerals of Mesopotamian catfish are shown in Table 1. The results showed significant differences between the four seasons ($p<0.05$) in major minerals.

Table 1. Seasonal changes in major mineral contents of Mesopotamian catfish (µg/g wet weight)

Seasons	Ca	P	K	Mg
Spring	95.93 ± 0.59^{c}	1469.45 ± 4.05^{b}	2955.67 ± 5.03^{c}	210.53 ± 0.47^{b}
Summer	103.56 ± 0.06^{d}	1557.25 ± 31.68^{c}	3132.67 ± 2.51^{d}	265.23 ± 1.07^{d}
Autumn	90.66 ± 0.66^{b}	1482.18 ± 53.99^{b}	2790.00 ± 3.00^{b}	254.07 ± 0.55^{c}
Winter	80.21 ± 0.55^{a}	1281.36 ± 33.63^{a}	2171.67 ± 4.72^{a}	179.20 ± 0.20^{a}
Avarage	92.59±9.81	1447.56±117.38	2762.50±418.00	227.26±39.79

Data are expressed as mean±SD of triplicate measurements Different superscripts in a column show significant differences between samples ($p<0.05$)

It is observed that during the summer period, Mesopotamian catfish showed the highest Ca(103.56 µg/g), P(1557.25 µg/g), K(3132.67 µg/g) and Mg(265.23 µg/g) levels. On the opposite, mineral content in winter were the lowest registered as 80.21 µg/g, 1281.36 µg/g, 2171.67 µg/g and 179.20µg/g respectively.

The results of seasonal proximate analysis in the flesh of Mesopotamian catfish are shown in Table 2. The results showed significant differences between the four seasons ($p<0.05$) in proximate composition.

Table 2. Seasonal changes in proximate composition of Mesopotamian catfish

Seasons	Crude Proteing/100g	Lipid g/100g	Moisture g/100g	Ash g/100g	Energy kcal/100g
Spring	17.54±0.14[a]	4.35±0.00[a]	77.15±0.00[a]	0.93±0.00[a]	109
Summer	17.44±0.00[b]	4.22±0.00[b]	77.39±0.00[b]	0.91±0.00[b]	108
Autumn	18.88±0.03[c]	4.35±0.00[a]	75.90±0.01[c]	0.83±0.00[c]	115
Winter	16.88±0.00[d]	6.56±0.00[c]	75.80±0.00[d]	0.67±0.00[d]	127
Avarage	17.68±0.84	4.87±1.12	76.56±0.82	0.84±0.11	114.75±8.73

Data are expressed as mean±SD of triplicate measurements Different superscripts in a column show significant differences between samples ($p<0.05$)

The highest crude protein value in Mesopotamian catfish was observed in autumn (18.88%) and the lowest in winter (16.88%). It was observed that the avarage protein content was 17.68%. Spawning and feeding habits are thought to be the main cause of the seasonal variations in major minerals and proximate composition in flesh of Mesopotamian catfish.

Ozyurt et al. [20] also reported variation in mineral content of *Sander lucioperca*, *Cyprinus carpio* and *Silurus glanis.* K contents of these species were 3547.0 µg/g, 3581.0 µg/g and 3059.0 µg/g respectively. Their Mg contents were 374.30 µg/g, 342.10 µg/g and 271.10 µg/g respectively. When the concentration of K and Mg in this study were compared with other some other freshwater fish species from Turkey, it was noted that *S. triostegus* had low quantities of K and Mg than *S. lucioperca*, *C. carpio* and *S. glanis*. Similarly, the avarage Ca and P contents in the present study were identified as lower than those reported previously for some freshwater species from Atatürk Dam Lake s uch as *Barbus grypus* and *Oncorhynchus mykiss* [21,22]. At the same time all seasons showed Ca values lower than 250 µg/g, which is the medium Ca value in fish as reported by Martinez-Valverde et al. [23].

The observed range of ash content Table 2 also indicated that the species is poor in minerals. Because the ash values is also lower (for *B. grypus* 1.06 g/100 g, *Mastacembelus mastacembelus* 1.38 g/100 g, *O. mykiss* 1.36 g/100 g*, S. lucioperca* 0.98 g/100 g, *C. carpio* 1.17 g/100 g and *S. glanis* 0.97 g/100 g*)* than that reported by other authors [23-25].

The maximum protein content (18.88%) were lower than or similar to the other economical freshwater fish species from Atatürk Dam Lake such as *B. grypus* (19.34%), *M. mastacembelus* (19.88%), *O. mykiss* (19.60%) [21,22,24] and other freshwater species such as *Brycon cephalus* (20.03%), *Brycon microlepis* (19.83%), *Brycon orbygnyanus* (18.94%) *S. lucioperca* (16.93-18.81%), *Channa lucius* (19.9%), *Perca fluviatilis* (18.43%), *Tinca tinca* (16.53-19.72%), *Vimba vimba tenella*, *Tilapia* spp. (18.02%) which have also been studied in Turkish inland waters [25-30].

The lipid content was identified to differ between 4.22-6.56% and the highest value was observed in winter (6.56%). Considering that, energy levels are associated with fat content, the energy value was also at the highest in the winter (127kcal/100g). Similar results are reported in *B. grypus* from Atatürk Dam Lake, [22] with the highest energ y energy and lipid content in winter period. On the other hand, the avarage lipid content (4.87%) was high in the present study when compared with other freshwater species from Atatürk Dam Lake such as *Barbus grypus* (4.04%), *M. mastacembelus* (2.10%), *O. mykiss* (4.43%) and other freshwater species from inland water of Turkey such as *T. tinca* (0.61%-

2.46%), *C. carpio* (0.88%), *S. lucioperca* (0.10-0.28%) and *S. glanis* (0.54%) *V. vimba tenella* (2.41%), *Tilapia* spp. (2.65%) [21-27].

4. CONCLUSION

The results of this study showed that Mesopotamian catfish from Atatürk Dam Lake (Turkey, Adiyaman) has rich lipid content, a low amount of major minerals and protein content when compared with other economical freshwater species from Turkey. However, the highest levels of the major minerals in Mesopotamian catfish (*S. triostegus*) were recorded in summer period.

ACKNOWLEDGEMENT

This work has been carried out with the financial support of Adiyaman University within the Research Project.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Kumaran R, Ravi V, Gunala B, Murugan S, Sundramanickam A. Estimation of proximate, amino acids, fatty acids and mineral composition of mullet (*Mugil cephalus*) of Parangipettai, Southeast Coast of India. Adv Appl Sci Res. 2012;3(4):2015-201.
2. Rudkowska I, Marcotte B, Pilon G, Lavigne C, Marette A, Vohl MC. Fish nutrients decrease expression levels of tumor necrosis factor-alpha in cultured human macrophages. Physiol. Genomics. 2010;40:189–194.
3. Lund EK. Health benefits of seafood; Is it just the fatty acids? Food Chem. 2013;140:413–420.
4. Khalili Tilami S, Sampels S. Nutritional value of fish: Lipids, proteins, vitamins, and minerals. Reviews in Fisheries Science & Aquaculture. 2018;26(2):243-253.
5. Ryan KO. Minerals Elements of Human Nutrition; 2009;24.
   Available: www.learningseed.com.
   (Accessed: 27.02.2013)
6. £uczyñska J, Toñska E, £uczyñski MJ. Essential mineral components in the muscles of six freshwater fish from the Mazurian Great Lakes (Northeastern Poland). Arch Pol Fish. 2009;17:171-178.
7. Nurnadia AA, Azrina A, Amin I, Mohd YAS, Mohd IEH. Mineral contents of selected marine fish and shellfish from the west coast of Peninsular Malaysia. Int Food Re J. 2013;20(1):431-437.
8. Fawole OO, Ogundiran MA, Ayandiran TA, Olagunju OF. Proximate and mineral composition in some selected fresh water fishes in Nigeria. Internet Journal of Food Safety. 2007;9:52-55.
9. Onyia LU, Milam C, Manu JM, Allison DS. Proximate and mineral composition in some Freshwater fishes in Upper River Benue, Yola, Nigeria Continental. J. Food Sci Tech. 2010;4:1–6.
10. Emmanuel BE, Oshionebo C, Aladetohun NF. Comparative analysis of the proximate compositions of Tarpon atlanticus and Clarias gariepinus from culture systems in South-western Nigeria. African Journal of Food, Agriculture, Nutrition and Development. 2011;11:6.
11. Vignesh R, Srinivasan M. Nutritional quality of processed head and bone flours of Tilapia (*Oreochromis mossambicus*, Peters 1852) from Parangipettai estuary, South East Coast of India. Asian Pac J Trop Biomed. 2012:S368-S372.
12. Adeniyi SA, Orjiekwe CL, Ehiagbonare JE, Josiah SJ. Nutritional Composition of three different fishes (*Clarias gariepinus, Malapterurus electricusand, Tilapia guineensis*). Pak J Nutr. 2012;11(9):793-797.
13. Olgunoglu İA, Artar E, Olgunoğlu MP. The fisheries situation and economic fısh species caught in Adiyaman province. Harran of University, J Agric. 2009;13(2):29-34 (in Turkish).
14. Oymak SA, Akın HH, Doğan N. Heavy metal in tissues of tor grypus from Atatürk Dam Lake, Euphrates River-Turkey. Biologia. 2009;64(1):151-15.

15. Cengiz Eİ, Ünlü E, Bashan M, Satar A, Uysal E. Effects of se asonal variations on the fatty acid composition of total lipid, phospholipid and triacylglicerol in the dorsal muscle of Mesopotamian catfish (*Silurus triostegus,* Heckel, 1843) in Tigris River (Turkey). Turk J Fish Aqua Sci. 2012;12:33-39.
16. AOAC (Association of Official Analytical Chemists Method 999.10. Official methods of analysis of AOAC. Internationalmethods 18 th. Ed. AOAC International, Gaithersburg, MD,USA; 2005.
17. AOAC (Association of Official Analytical Chemists) Official Methods of Analysis of the Association of Official Analytical Chemistry. 16th Edn., AOAC International, Washington, USA. 1995:1141.
18. Watt BK, Merrill AL. Composition of foods: Raw, processed and prepared (Agriculture Handbook No. 8.). United States Department of Agriculture. Washington D.C. 1975:190.
19. Bozkurt Y, Bekcan S, Çakıro ğulları GÇ. Seasonal variation and meat composition of Bleak (*Alburnus orontis*, Sauvage 1882) Ankara University Faculty of Agriculture. J Agric Sci. 2006;12(1):70-73.
20. Özyurt G, Polat A, Loker GB. Vitamin and minera l content of pike perch (*Sander lucioperca*), common carp (*Cyprinus carpio*), and European catfish (*Silurus glanis*). Turk J Vet Anim Sci. 2009;33(4):351-356.
21. Çelik M, Gökçe MA, Basusta N, Küçükgülmez A, Ta sbozan O, Tabakoglu SS. Nutritional quality of rainbow trout (*Oncorhynchus Mykiss*) caught from the Atatürk Dam Lake in Turkey. J Muscle Foods. 2008;19:50–61
22. Olgunoglu İA, Olgunoglu MP, Artar E. Seasonal changes in biochemical composition and meat yield of Shabut (*Barbus grypus*, Heckel 1843). Iran J Fish Sci. 2011;10(1):183-189.
23. Martinez-Valverde IM, Periago MJ, Santaella M, Ros G. The content and nutritional significance of minerals on fish flesh in the presence and absence of bone. Food Chem. 2000;71:503-509.
24. Olgunoglu İA. Determination of the fundamental nutritional components in fresh and hot smoked spiny eel (*Mastacembelus mastacembelus*, Bank and Solander, 1794) Sci Res Essays. 2011;6(31):6448-6453.
25. Dikel S, Çelik M. Body and nutritional composit ion of Tilapia (*Tilapia* spp.) from the Southern Seyhan River. Turk J Vet Anim Sci. 1998;22:517–520.
26. Diler A, Becer AZ. Chemical Composition and meat yield of Vimba (*Vimba vimba tenella* (Nordmann, 1840) in Karacaören I Dam Lake. Turk J. Vet. Anim. Sci. 2001;25:87-92.
27. Olgunoglu İA, Polat A, Var I. Chemical and Sensory Changes of Pike-Perch (*Sander lucioperca* Bogustkaya & Naseka, 1996) fillets during frozen storage (-18ºC). Turk J Vet Anim Sci. 2002;26:879-884.
28. 25. Zencir Ö, Korkmaz A ß. Meat yield and body composition of Tench (*Tinca tinca* L., 1758) in Lake Bey□ehir. Ankara University Faculty of Agriculture, J Agric Sci. 2004;10(4):474-480.
29. Çelik M, Diler A, Küçükgülmez A. comparison of the proximate compositions and fatty acid profiles of zander (*Sander lucioperca*) from two different regions and climatic conditions. Food Chem. 2005;92:637–641.
30. Gökçe MA, Tasbozan O, Tabakoglu SS, Çelik M, Oz can F. Basusta A. Proximate composition and fatty acid profile of shabbout (*Barbus grypus,* Heckel) caught from the Atatürk Dam Lake, Turkey. J Food Agric Environ. 2011;9(2):148-151.

Biography of author(s)



Mine Perçin Olgunoglu
Department of Aquaculture and Fisheries Program, Kahta Vocational Training School, Adıyaman University, Turkey.

Research and Academic Experience: Associate Professor of Fisheries Sciences at the Department of Aquaculture and Fisheries Program, Kahta Vocational Training School, Adiyaman University, 02400 Turkey.

Research Area: Basic Sciences on Fisheries, Biochemistry, Environmental Pollution.

Number of Published papers: One Book, more than 30 published scientific articles.

Special Award (If any): Awards of Publication incentive.



Ilkan Ali Olgunoğlu
Department of Aquaculture and Fisheries Program, Kahta Vocational Training School, Adıyaman University, Turkey.

Research and Academic Experience: Associate Professor of Fisheries Sciences at the Department of Aquaculture and Fisheries Program, Kahta Vocational Training School, Adiyaman University, 02400 Turkey.

Research Area: Fish Processing Technology, Biochemistry, and Food Science and Quality Technology.

Number of Published papers: One Book, one Book Chapter and more than 40 published scientific articles (H-index 9).



Mustafa Göçer
Department of Aquaculture and Fisheries Program, Kahta Vocational Training School, Adıyaman University, Turkey.

Research and Academic Experience: 24 years.

Research Area: Fishing and Processing Technology.

Number of Published papers: 20

© Copyright (2021): Author(s). The licensee is the publisher (Book Publisher International).

DISCLAIMER
This chapter is an extended version of the article published by the same author(s) in the following journal.
Annual Research & Review in Biology, 4(16): 2628-2633, 2014.

Chapter 10
Print ISBN: 978-93-90516-93-3, eBook ISBN: 978-93-90516-94-0

Taxonomic Keys of Mayflies in the Palni and Cardamom Hills of Western Ghats, Southern India

S. Barathy[1*], T. Sivaruban[2] and Pandiarajan Srinivasan[2]

DOI: 10.9734/bpi/rrab/v5/6233D

ABSTRACT

A key to ephemeropteran nymphs belonging to 8 families, 30 genera and 48 species from the Palni and Cardamom hills of the Western Ghats in Kerala and Tamil Nadu states is presented in this chapter. Sampling was done at 30 sites. Baetidae and Leptophlebiidae diversity was higher compared to Ephemeridae and Ephemerellidae. Mayflies endemic to the Palni and Cardamom hills include *Ephemera (Aethephemera) nadinae, Klugephlebia kodai* and *Indoganodes jobini*. This work serves as a taxonomic primer for doing further research on mayfly taxonomy, biology, biogeography and ecology and it will also aid Bio monitoring programs.

Keywords: Key; Baetidae; gills; setae; terga; endemism.

1. INTRODUCTION

Freshwater ecosystems are being subjected to anthropogenic disturbances throughout the world in recent times [1]. Benthic macro invertebrates are among the most important components of freshwater ecosystem; they are cosmopolitan in distribution and occur in several substrate types in freshwater environments [2]. The distribution of aquatic insects is influenced by their interactions of habitats, physico-chemical attributes, structural and hydrological characteristics and anthropogenic stress [3, 4].

Mayfly (Order: Ephemeroptera) larvae are living in fresh water habitats such as lakes, streams, ponds and rivers. Larvae of May fly commonly occurs in under stone, decaying leaf litters, wood logs, etc. The mayfly larvae are inhabited in water bodies while sub imagoes and imagoes are spending their life in terrestrial habitats. Larvae of mayfly size, shape and body plan are different from species to species and they are also slender, soft bodied creatures lacking wings and have feathery external gills along the sides or on the abdominal region. Their flattened head helps them to adhere to the rocks in fast flowing water. Larvae have 3 or 2 cerci in the terminal region of abdomen. Structure and location of gills on the larvae is essential characteristic feature for species identification.

Mayfly larvae act as an ecosystem servicer, nutrient cycler and bio indicator in fresh water bodies. These larvae are of different feeding categories such as grazers, scraper, filter feeder, predators, etc. Identification keys are very important for the identification and distribution of taxa. The main aims of this larval keys are for i) identification of new mayfly taxa, ii) collection of scientific data from this taxa and iii) identify the endemic fauna in Palni and Cardamom hills in the Western Ghats. Mayflies are recognized as potential bio indicators of good water quality and they have not widely been studied in the Indian subcontinent [5]. The reason for selecting Palni and Cardamom hills for the study is because they are the part of the Western Ghats which is one of the hot spots in the World. Nowadays, mayflies of these sites are facing more threat and extinctions because of human interference. This generic key will definitely be making awareness among budding scientist and conservation of these aquatic creatures.

[1]Department of Zoology, Fatima College, Madurai-625018, India.
[2]PG& Research Department of Zoology, The American College, Madurai-625002, India.
**Corresponding author: E-mail: barathyruban@gmail.com;*

2. MATERIALS AND METHODS

2.1 Study Site

The present work was carried out from 2018-2019 in 30 streams of Palni and Cardamom hills (Table 1) of the Western Ghats in Southern India.

2.2 Sampling and Collection of Insects

Mayfly nymphs are collected using 1cm wide Kick-net, D-net and handpicking. All insects were picked and preserved in 80 percent ethanol and identified in the laboratory. Collected specimens were studied with the aid of a stereo microscope and identified with the help of a recent field guide [6] and appropriate taxonomic literature. Identification of all species was confirmed by the experts in this field.

Table 1. Details of the 30 Study sites

S. No	Name of the study site	Abbreviation	Stream order	Altitude (m)	Latitude (N°)	Longitude(E°)
1	Oothu	Ooth	3	1300	10º12'	77º26'
2	Perumalmalai	Peru	2	1400	10º18'	77º33'
3	Kurusadai	Kuru	2	1700	10º20'	77º28'
4	Ghandhi nagar	Gand	2	1600	10º18'	77º27'
5	Silver cascade	Silv	2	1700	10º12'	77º28'
6	Vattakanal	Vatt	2	1000	10º11'	77º25'
7	Fairy falls	Fair	3	290	10º13'	77º27'
8	Bear Shola falls	Bear	2	300	10º14'	77º27'
9	Fern hill falls	Fern	3	122	10º12'	77º20'
10	Pillar rock	Pill	1	2250	10º17'	77º28'
11	Near pillar rock	Near pill	1	2255	10º12'	77º30'
12	Pambar stream	Pamb	2	2248	10º13'	77º28'
13	Dhobikana	Dhob	3	2075	10º24'	77º24'
14	GundarFalls	Gund	2	2200	10º14'	77º26'
15	Poomparai	Poom	2	2133	10º13'	78º16'
16	Kounchi	Kouc	2	2360	10º29'	77º30'
17	Kurangani up	Kura-up	2	2410	11º00'	77º50'
18	Kurangani down	Kura-down	2	2345	11º00'	77º45'
19	B. L. Rave	B.L.Rave	1	1250	10º11'	77º25'
20	Poonthampanai	Poon	1	1300	11º12'	77º26'
21	Santhamparai near bridge	San-bridge	2	1350	11º00'	77º52'
22	Santhamparai near SDA school	San-SDA	4	1400	11º13'	77º28'
23	Mattupetty Dam stream	Matt	2	1700	11º16'	77º29'
24	Anayirankal stream	Anai	2	1950	11º18'	77º31'
25	Aranmanaiparai	Aran	3	2050	11º21'	77º33'
26	Popparai	Popp	4	1785	11º44'	77º26'
27	Bodimettu	Bodi	2	1500	11º15'	77º24'
28	Thoovanam falls	Thuv	3	1550	11º15'	77º15'
29	Nayamakkadu falls	Naya	2	2197	11º20'	77º10'
30	Chinnakanal falls	Chin	1	1800	11º27'	77º30'

3. RESULTS AND DISCUSSION

3.1 Mayfly Diversity

A total of 1255 mayfly nymphs were collected during the period of 2018-2019 from the Palni and Cardamom hills comprising eight mayfly families, 30 genera and 48 species. Baetidae was the most diverse family with 17 species followed by Leptophlebiidae with 14 species. The lowest diversity

occurred in Ephemeridae and Ephemerellidae, each with one species. The highest numbers of nymphs collected were of *Choroterpes alagarensis* (Leptophlebiidae) and *Tenuibaetis frequentus* (Baetidae). *Potamanthellus ganges* (Neoephemeridae) was the least collected species.

3.2 Endemism

Western Ghats is one of the most noted biodiversity hotspots of the world and its fauna exhibits a high level of endemicity. Mayflies in the Palni and Cardamom hills show endemicity to some extent, the mayflies that are endemic to the Palni and Cardamom hills are listed in Table 2.

Table 2. List of endemic taxa present in Palni and Cardamom hills

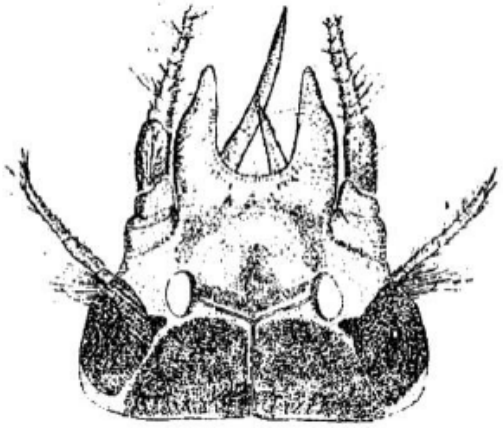
S. No	Family	Taxa
1	Ephemeridae	*Ephemera (Aethephemera) nadinae*
2	Leptophlebiidae	*Klugephlebia kodai*
3	Teloganodidae	*Indoganodes jobini*

Key to families of mayflies based on mature nymph of Palni and Cardamom hills:

1. Presence of seven simple gills including gill 1; lateral ocelli located above lateral areas of epicranial suture; ventral orientation of dorsal lobe on the apice of the femora……..……**Baetidae**
   -Gills variously developed, gill 1 either present or absent; lateral ocelli connecting with lateral branches of the epicranial suture or situated below the suture; no ventral orientation of dorsal lobe on the apice of the femora……**2**
2. Mandibular tusks present, tusks curved outwards, inner edges convex; tibial extension on posterior area of legs ……..**Ephemeridae**
   -Mandibular tusks absent……..……..……..**3**
3. Gills absent on abdominal segment 2 ……..……..…..**Ephemerellidae**
   -Gills present on abdominal segment 2……..**4**
4. Forewing pads freely extending beyond fusion to thorax for more than half their length; gill 2 not operculate……..……..**5**
   - Forewing pads freely extending beyond fusion to thorax for less than half their length; gill 1 never operculate, operculate gills on abdominal segment 2 present or absent……..…..**6**
5. Head capsule mostly semi-circular and dorsoventrally flattened, gills 1-6 possessing plate-like dorsal lamella with a fibrilliform ventral portion, gill 7 lamellate without fibrilliform portion; no prominent brush of setae in the apicolateral margin of the maxilla ……..……**Heptageniidae**
   -Head capsule mostly quadrangular or rectangular, gill 1 sometimes simple, unilamellate, lanceolate or filamentous and rudimentary; gills 2-7 bilamellate; prominent brush of dense setae in the apicolateral margin of the maxilla……..**Leptophlebiidae**
6. Gills on abdominal segment II meeting along the midline, median caudal filament covered with dense setae on outer and inner margins; the lateral filaments with setae only on the inner margins.....**Neoephemeridae**
   - Gill on abdominal segment II not as above……..……**7**
7. Abdominal gills on segment II overlapping with setae or spatulate spines on posterior margin at midline and presence of Y-shaped ridges on the dorsum; terminal filament present or absent and abdomen wider than thorax; gill 1 always filamentous……..……..…**Caenidae**
   -Gill 1 present or absent and if it is present, it is filamentous; gill 2 operculate, semi-operculate, or not operculate; even if it is operculate, it is smaller than above and it is generally oval in shape; lack of Y-shaped ridges on dorsal surface and it is never overlapping, posterior margin of mesonotum with prominent V shaped notch and generally lobed basally ……..……..**Teloganodidae**

Key to genera and known species of mayflies from family Ephemeridae:

1. Well-developed mandibular tusks; abdominal gill 1 with two lobes mostly of equal size.....***Ephemera***.....**a**
   a. Mandibular tusks asymmetrical; right one is shorter (Fig. 1a).…….subgenus: ***Aethephemera***.....**i**
      i. Frontal process in the golden brown; tibia in the dorsal surface of prothoracic legs entirely spuriferous; Meta thoracic legs tibial process is densely covered with golden brown setae. (Fig. 1b)……..***Ephemera (Aethephemera) nadinae***



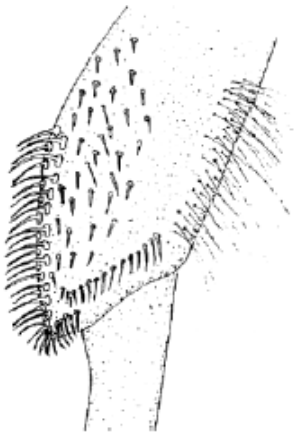


Fig. 1a. **Fig. 1b.**
(after Balasubramanian et al. [7])

Key to genera and known species of mayflies from family Ephemerellidae:

1. Gill III is enlarged and semi-operculate, covering the other gills (Fig. 2) ……***Torleya***.....**i**
   i. Head capsule distinctly have a row of spatulate setae along anterior margin; setae of median caudal filament recumbent and 2-3x the length of the filament segment……..……..*****Torleya nepalica***

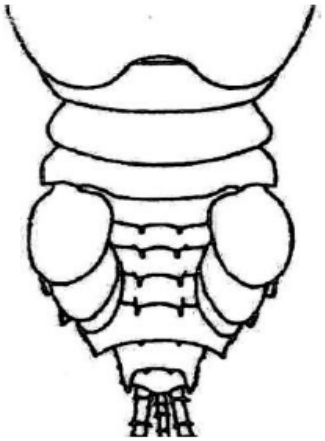


Fig. 2.

Key to genera and known species of mayflies from family Neoephemeridae:

1. Dense setated mouthparts, lateral expansions of the pronotum and mesonotum are not well developed; caudal filaments having rows of long setae……..……***Potamanthellus***.....**i**
   i. Diagonal ridge on operculate gills (Fig. 3); distinct postero-median tubercle on terga 6–8 and dorsal forefemur lacks row of setae……..……***Potamanthellus ganges***



Fig. 3.

- Posteromedian tubercles on terga 1–2 and 6–8 distinct (Fig. 4a); strongly developed rows of hair like setae and dorsal fore femur have transverse row of setae (Fig. 4b)……..…..***Potamanthellus caenoides***



Fig. 4a. **Fig. 4b.**
(after Selvakumar et al. [8])

Key to genera and known species of mayflies from family Caenidae:

1. Clypeus with anterior margin produced; foretibiae with long filtering setae……***Clypeocaenis***……**i**

- Clypeus with anterior margin not produced; foretibiae lacks long filtering setae……..……..……..……..…***Caenis* sp**

  i. Posterior margin of clypeal protrusion have two long setae with hairs (Fig. 5a); setae shorter than the width of femora on the margins of middle and hind region of femora; femora of foreleg have a group of four setae (Fig. 5b); presence of pointed spines in the posterior margin of terga I and II and lack of scales and spines in terga III – VII……..…***Clypeocaenis bisetosa***

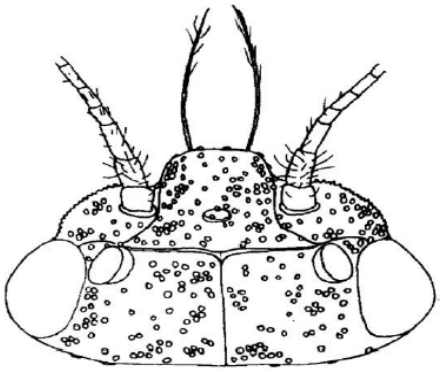


Fig. 5a.



Fig. 5b.

(after Soldan, [9])

- Anterior margin of the clypeal protrusion with 10-15 long plumose setae; setae on the margins of middle and hind region of femora longer than femoral width, transverse row of stout spines on forefemur (Fig 6), spines lacking on medial projection of tergum II; blunt teeth on the posterior margins of terga VIII and IX……..……..……...***Clypeocaenis multisetosa***

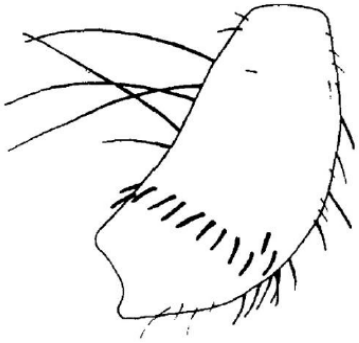


Fig. 6.
(after Soldan, [9])

Key to genera and known species of mayflies from family Leptophlebiidae:

1. Terga extending around to the ventral surface of the abdomen on segments III-VII; slender gills arise ventrally (Fig. 7)……..……...***Isca sp***



Fig. 7.

- Terga extending to lateral sides of abdomen, all gills on the abdomen lateral or dorsal.....**2**

2. Presence of gills on abdominal segments 1–6……..….***Notophlebia***…….**i**

i. Rectangular shaped gills; tibiae on the prothoracic legs are interspersed with plumose spines and hair laterally (Fig. 8a); tibiae of other legs hairy and basal 4 denticles on claws are distinctly developed from remaining denticles, Dilatognathus-type mouth apparatus (Fig. 8b)……....***Notophlebia jobi***



Fig. 8a.

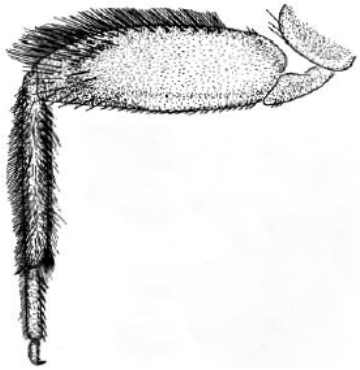


Fig. 8b.

(after Sivaramakrishnan & Peters, [10])

- Abdominal gills narrower; third segment of maxillary palp have moderately long, slender setae, situated densely and irregular; third segment of labial palp with long filtering setae moderately developed on dorsal side and directed apically-inward; non- dilatognathus mouth apparatuses (Fig. 9).....***Notophlebia ganeshi***

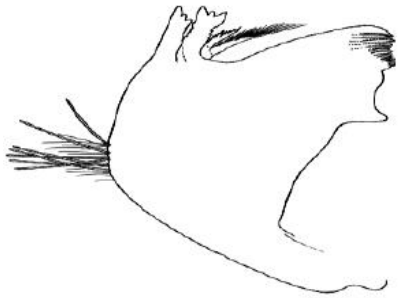


Fig. 9.

- Gills present on abdominal segments 1–7……..……....**3**

3. Abdominal gills (3–5) with a fringed margin or with filamentous processes……...**4**
- Middle abdominal gills not as above……...**5**
4. Middle abdominal gills plate like, broadly oval and margins fringed with filamentous processes……..……***Thraulus***
   i. Mandible lateral setae sparse, thin clump near middle of lateral margin, thinning posteriorly; labial palpi segment 3 with row of long thin lateral setae and 6-7 large dorsal setae; presence of 10-13 denticles in claw with decrease in size apically; gills on the segment 1 with a lanceolate dorsal lamella and an ventral lamella looks oval (Fig. 10)…….....***Thraulus gopalani***

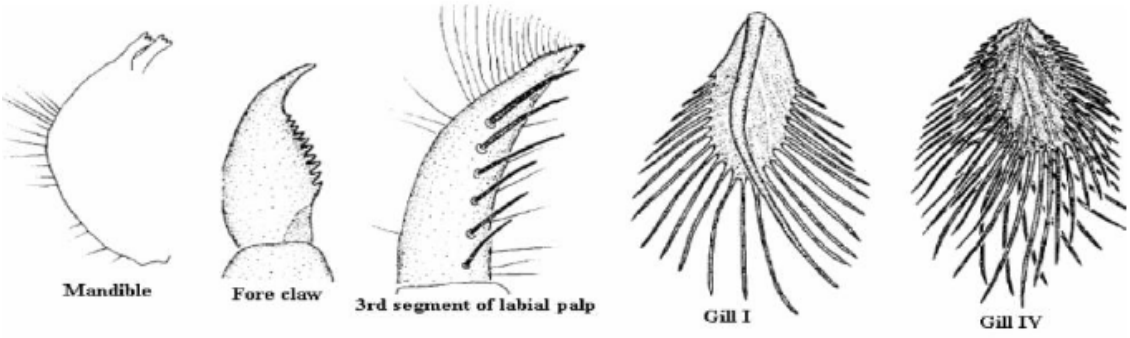


Fig. 10.
(after Grant & Sivaramakrishnan, [11])

- Middle abdominal gills plate like, irregular, unevenly fringed with broad filamentous processes……..……***Petersula***
   a. Dorsum of thorax yellowish brown, washed with dark brown at margins; Venter pale; marks of nota as in figure given in the below, posterior margin of terga 1-10 with a narrow, brownish-black, transverse band; sterna pale, posterior margin of all abdominal terga with a row of fine spines; presence of postero-lateral spines on segments 3-9, spines progressively larger posteriorly, median caudal filament little longer than the cerci (Fig. 11)……...***Petersula courtallensis***

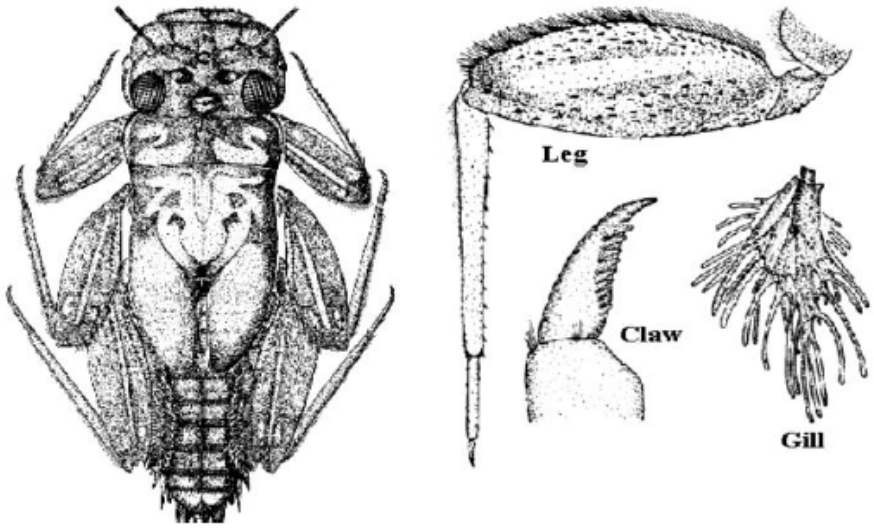


Fig. 11.
(after Sivaramakrishnan, [12])

5. Leaf-like middle abdominal gills; three filamentous processes present on the apex of gills 1–7, median one is longer than that of lateral processes……***Nathanella***……..…..**i**
   i. Median projection of abdominal gills broad and approximately twice length of laterals; tracheation in gills uniformly disturbed (Fig. 12b); distal, irregular light brown maculae on femora of legs (Fig. 12a)…….***Nathanella indica***

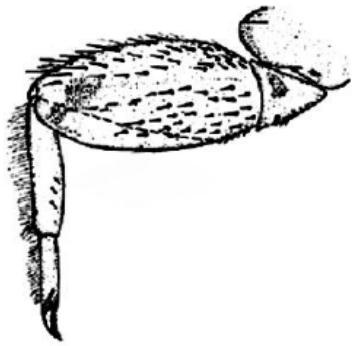


Fig. 12a.



Fig. 12b.

(after Sivaramakrishnan et al. [13])

- Median projection of abdominal gills narrow and approximately 1-1/2 times length of laterals; main trunk of tracheae of gills forked near distal half of lamellae (Fig. 13b); medial and distal, irregular black maculae on femora of legs (Fig. 13a)……...***Nathanella saraswathiae***

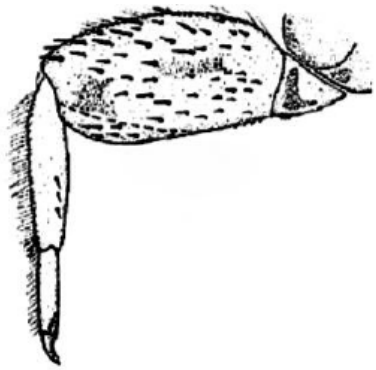


Fig. 13a.



Fig. 13b.

(after Sivaramakrishnan et al. [13])

- Middle abdominal gills plate like; gill apex not as above……..……..**6**

6. Lamellae of gills 2–7 trifurcate……***Choroterpes***……**a**
   a. Gills 1–7 monolamellate……subgenus……***Choroterpes*** (***Monochoroterpes***)
   - Gills 1–7 bilamellate……..……..**b**
   b. Middle abdominal gills terminated in 3 slender, subequal processes……. subgenus……***Choroterpes (Euthraulus)***…….....**i**
      i. Tarsal claw with 15 denticles (Fig. 14)……...***Choroterpes alagarensis***

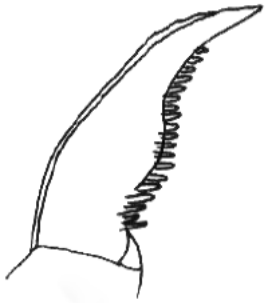


Fig. 14.

- Tarsal claw with 12 or 13 denticles (Fig. 15b)……..……..**ii**

ii. Paraprocts smooth without pores and their inner and outer margins bare (Fig. 15a)……..……***Choroterpes nambiyarensis***



Fig. 15a.

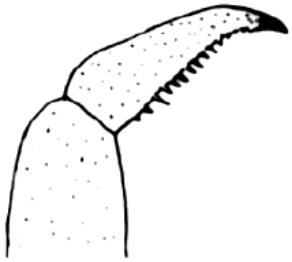


Fig. 15b.

- Paraprocts smooth with pores and presence of small triangular spines in inner margin with and long setae on the outer margin (Fig. 16)……..…....***Choroterpes kalladaensis***



Fig. 16.
(after Rekha et al. [14])

- Middle abdominal gills terminated in 3 processes, with median projection longer than laterals……subgenus……..…..***Choroterpes***…….……..**i**

i. Abdominal gill 1 slender with dorsal and ventral portions (Fig. 17); median projection of gills 2–7 plate-like and markedly larger and longer than laterals and labrum with three transverse rows of setae on dorsal surface, middle row without setae medially; anteromedian margin of labrum with a deep U-shaped ventral incision……***Choroterpes petersi***

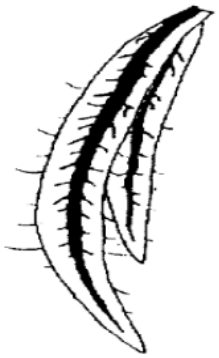


Fig. 17.
(after Tong & Dudgeon, [15])

- Lamellae of gills 2–7 not trifurcate……..…..**9**

9. Claw denticles progressively larger apically; apical denticle much larger……***Edmundsula***…….……**i**

i. Gills with abdominal segments I - VII; gill I dorsal and ventral regions are slender and lanceolate with few tracheae; dorsal and ventral areas of lamellae in the gills II - VII are lanceolate, long, and smoothly tapered near apex; segment 3 of labial palpi has a row of heavy spines on inner dorsal margin; smoothly curved outer margin of mandibles with 8 to 10 long hairs on median area of outer margin (Fig. 18)….....***Edmundsula lotica***

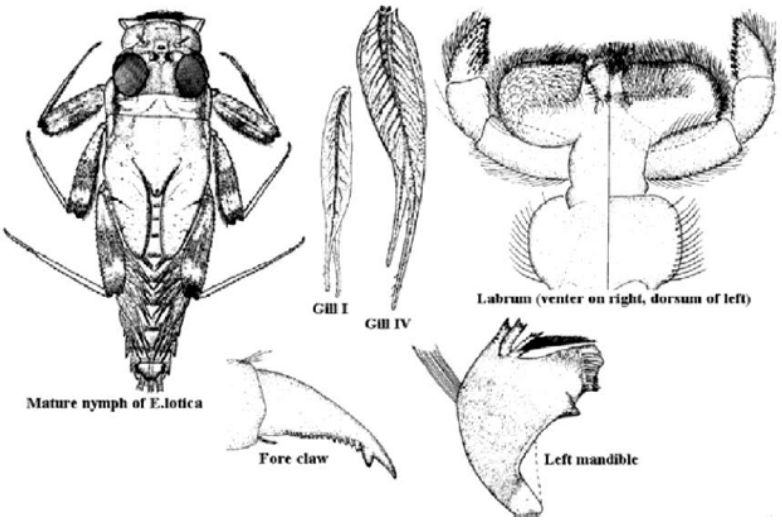


Fig. 18.
(after Sivaramakrishnan, [16])

- Apical denticle not much larger…….....**10**

10. Gills 1–7 similar, lamellar dorsal and ventral regions slender, tracheae branched……***Indialis***……..…**i**

i. Body dark brown, sutures and sterna paler, markings on abdomen as in figure. Legs and caudal filaments look pale. Gills hyaline, tracheae black (Fig. 19)……..……..…***Indialis badia***

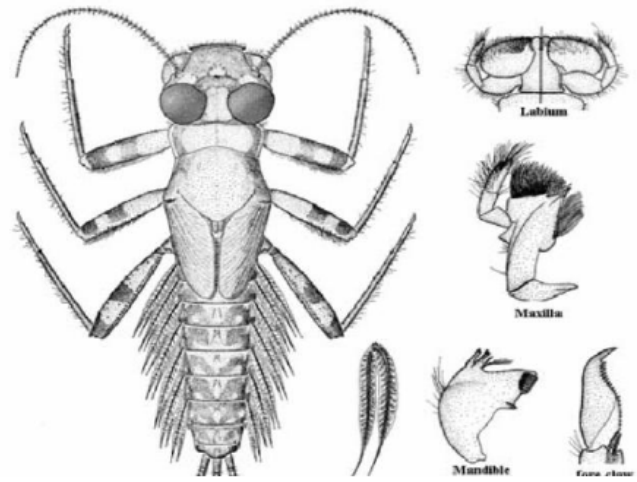


Fig. 19.
(after Peters & Edmunds, [17])

- Gills 1–7 not similar; lamellar dorsal and ventral portions of gill 1 slender and lanceolate with trachea branched; gills 2–7 lamellar dorsal and ventral portions wider and lanceolate and tapering near apex (Fig. 20b)……..***Klugephlebia***……..….**i**
  i. Fore and mid femora with a regular row of long, thin setae on outer margin; denticles on claws progressively larger apically; length of the labrum more than half of the width, lateral lobes rounded , anteriomedian emargination deeply cleft (Fig. 20a), apparently with 2 denticles; proximal transverse setal row laterally curved distally; maxillary palp short, with long setae on third segment and third segment of labial palp with 5–6 thick, dorsal surface have spine-like setae, inner and outer margins with short, thin setae…..***Klugephlebia kodai***



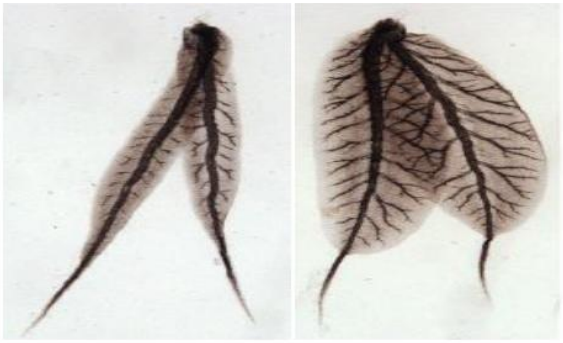


Fig. 20a. **Fig. 20b.**
(after Selvakumar et al. [18])

Key to genera and known species of mayflies from family Teloganodidae:

1. Terminal filament length subequal to length of cerci.....***Indoganodes***.....**i**
   i. Gills on segments II–VI (Fig. 21a); gill 1 absent; tarsal claw hooked (Fig. 21b), bearing 4 small denticles medially.....***Indoganodes jobini***



Fig. 21a.

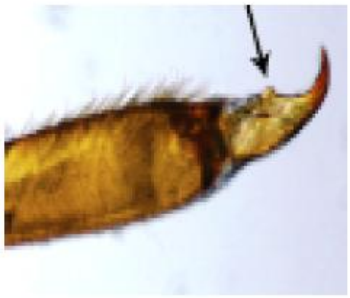


Fig. 21b.

(after Selvakumar et al. [19])

- Median caudal filament reduced or apparently absent.....2

2. Gills on segments II–IV (Fig. 22a); prostheca of left mandible reduced, but with several well-developed seta-like projections.....***Derlethina***.....**i**

i. Gill 4 incised; outer margin of hind femora not concave; and prostheca of left mandible reduced (Fig. 22b), but with several well-developed seta-like projections.....***Derlethina tamiraparaniae***



Fig. 22a.



Fig. 22b.

(after Selvakumar et al. [20])

- Gills on segments II–V or II–VI; prostheca of left mandible not reduced, without seta-like projections.....3

3. Gills present on segments II–VI (Fig. 23a); forefemur without a row of transverse setae on dorsal face; teeth of inner incisor of left mandible similar in size.....***Teloganodes***…....**i**

i. Outer margin of the forefemora with a row of thick setae; two clusters of thin setae on apex (Fig. 23b).....***Teloganodes sartorii***



Fig. 23a.



Fig. 23b.

(after Selvakumar et al. [20])

- Outer margin of forefemora with a row of thin setae.....**ii**

ii. Outer margin of the forefemora with a dense row of thin setae(Fig. 24b) and without a row of thick setae; without two clusters of thin setae on apex (Fig. 24a).....***Teloganodes kodai***



Fig. 24a.

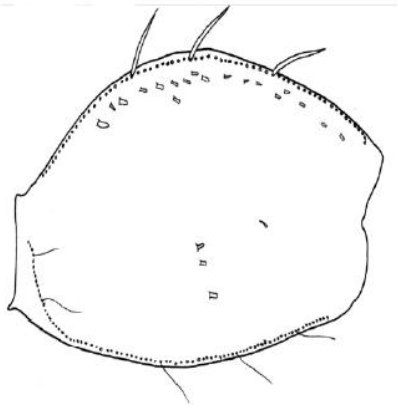


Fig. 24b.

(after Sartori et al. [21])

- Presence of dense row of thin setae and scattered thick and pointed setae on Outer margin of forefemora (Fig. 25a); Labrum ca. 3 times wider than long; outer margin of mandibles with one stout seta (Fig. 25b); outer margin of forefemora with 12–15 thick and pointed setae.....***Teloganodes insignis***

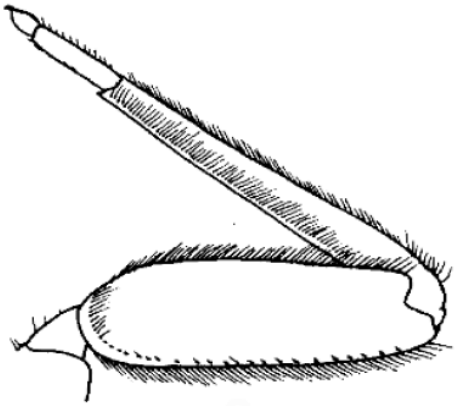




Fig. 25a. **Fig. 25b.**
(after Wang & McCafferty, [22])

- Abdominal gills present on segments II–V (Fig. 26a); forefemur with a row of transverse setae on dorsal face; teeth of inner incisor of left mandible dissimilar.....***Dudgeodes***.....**i**
  i. Long and stout pointed setae across dorsal face of transverse row in forefemur; labial palp segments 3 ca. 2.5 times as long as wide (Fig. 26b).....***Dudgeodes palnius***



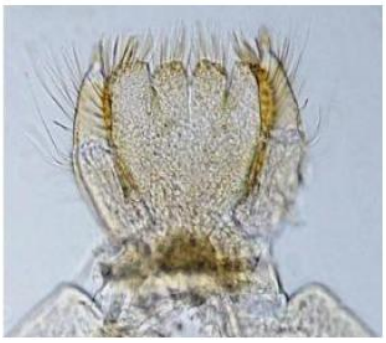


Fig. 26a. **Fig. 26b.**
(after Selvakumar et al. [20])

- Long and pointed setae across dorsal face of transverse row in forefemur (Fig. 27a); labial palp segment 3 ca. 2.7 times as long as wide (Fig. 27b).....***Dudgeodes bharathidasani***



Fig. 27a.

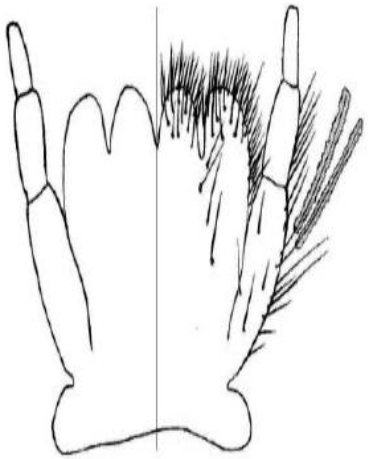


Fig. 27b.

(after Anbalagan et al. [23])

Key to genera and known species of mayflies from family Heptageniidae:

1. Absence of median caudal filament……..***Epeorus***……..……..**i**
   i. Abdominal terga yellowish brown, segment II–VIII have pair of sub median bars and pair of sub median spines and segment I–X have a row of sparse setae on posterior margin (Fig. 28a); posterolateral spines on abdominal segment II–VII, progressively longer and curved inwards posteriorly (Fig. 28b); and lamellate portion in the posterior edge of gill 1 bluntly pointed……..***Epeorus petersi***

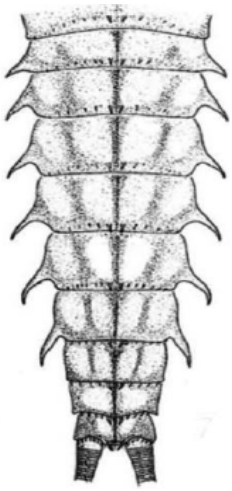


Fig. 28a.



Fig. 28b.

(after Sivaruban et al. [24])

- Presence of median caudal filament……..……..……..**2**
  2. Lack of long, fine setae at articulations in the caudal filaments; Terminal segment of labial palps have patch of long setae projecting medially; head capsule slightly thickened anteriorly……***Afronurus***…….……....**i**

i. Gill leaflets on abdominal segment 5 without acutely pointed apical elongation; femora of legs with scattered pale maculae and median, transverse, zig-zag pale yellow band (Fig. 29)…….***Afronurus Kumbakkariensis***

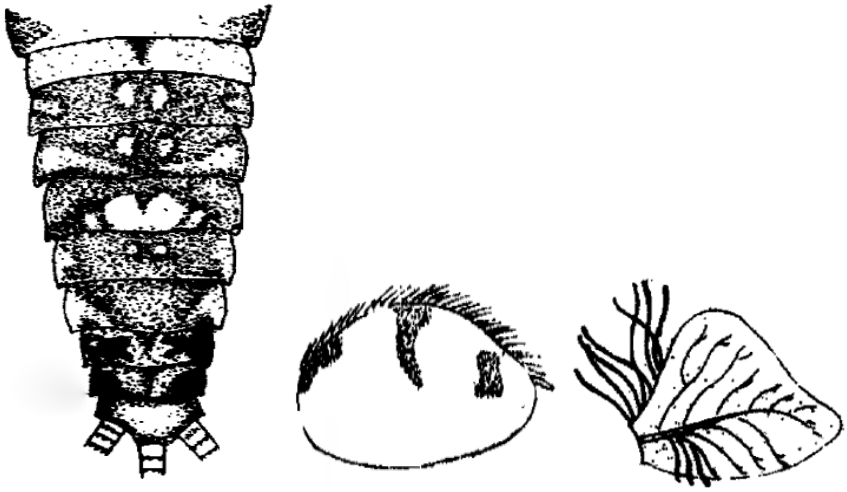


Fig. 29.
(after Venkataraman & Sivaramakrishnan, [25])

- Head capsule distinctly thickened anteriorly; abdominal posterolateral spines large; glossae subquadrate; femora and head capsule lacks numerous black dots… ***Thalerosphyrus***……...**i**

i. Gill I asymmetrical, ovoid and 1.5 times as long as wide, presence of yellowish brown with a median zig-zag pale yellow bands in femora of all legs; yellowish brown lateral filaments, caudal filaments are interspersed with blackish brown segments (Fig. 30)……...***Thalerosphyrus flowersi***

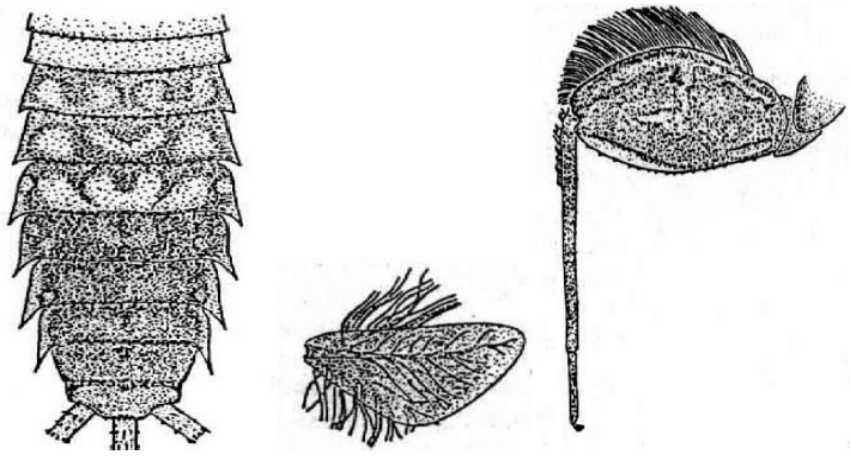
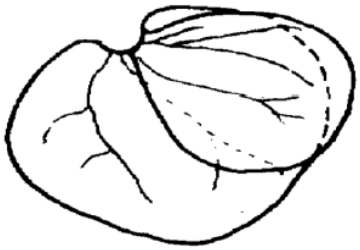


Fig. 30.
(after Venkataraman & Sivaramakrishnan, [26])

Key to genera and known species of mayflies from family Baetidae:

1. Gill lamellae of segments 1-6 double and the seventh gill single……...***Cloeon***……...**i**

i. Abdominal gills double(Fig. 31a); outer apical edge of 3rd segment of labial palpus well developed and inner margin S-shaped(Fig. 31b).....***Cloeon bimaculatum***



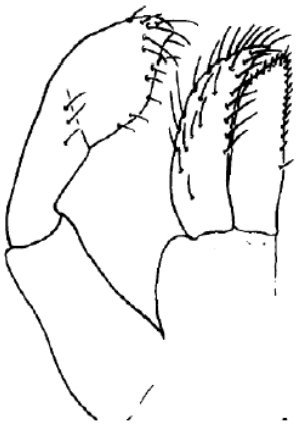


Fig. 31a. **Fig. 31b.**
(after Muller-Liebenau & Hubbard, [27])

- All gills have lamellae single,……..……**i**
  i. Abdominal gills single (Fig. 32a); outer apical edge of 3rd segment of labial palpus not extremely developed, inner margin bulging inwardly somewhat in the middle (Fig. 32b).....***Procloeon regularum***



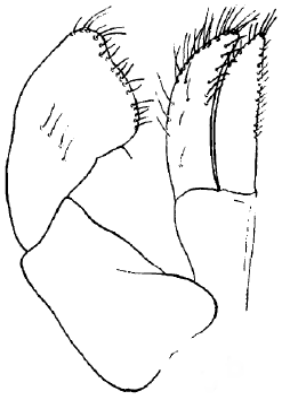


Fig. 32a. **Fig. 32b.**
(after Muller-Liebenau & Hubbard, [27])

2. Head and thorax laterally flattened, hind wing pads and gill 1 greatly reduced (Fig. 33a); abdominal tergal scale bases trapezoid; terminal filament shorter than lateral cerci; paraproct prolonged, right prostheca of mandible slender and pointed; glossa with transverse row of bristles on dorsal surface near apex……....***Indobaetis***……....**i**
   i. Maxillary palp reaching beyond galea-lacinia, without indentation on inner margin near apex (Fig. 33b), well developed lobe on the mesial margin in the second segment of labial palp; gills on segments 1–7.....***Indobaetis michaelohubbardi***



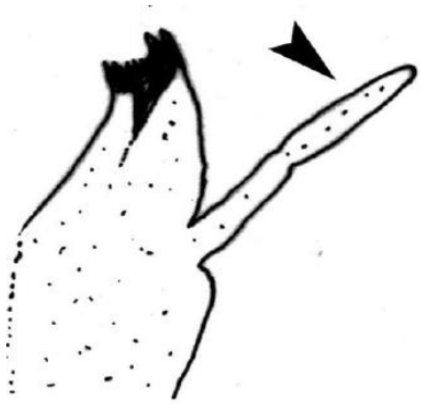


Fig. 33a. **Fig. 33b.**
(after Selvakumar et al. [28])

- Anterior margin of head capsule distinctly thickened long, sparse setae on the dorsal margin of femora; In between the prostheca and mola of right mandible, spine-like setae present; prostheca of right mandible elongate and bifid……..…***Centroptella***……...……**i**
(* Kluge N. J et al. [29], made revisions in the nomenclature of *Centroptella* genera and replace generic name *Bungona* into *Centroptella* because of its senior generic name)

i. Tarsal claws dentate; posterior marginal spines of tergite IX with broadly interrupted medially……..……..……**ii**

- Tarsal claws edentate; posterior marginal spines of tergite IX contiguous and complete…….....…**iv**

ii. Hindwing pads completely absent; Abdominal tergite I uniformly yellow-white; tergites IX–X with spines on posterior margin (Fig. 34)…….***Centroptella similis***

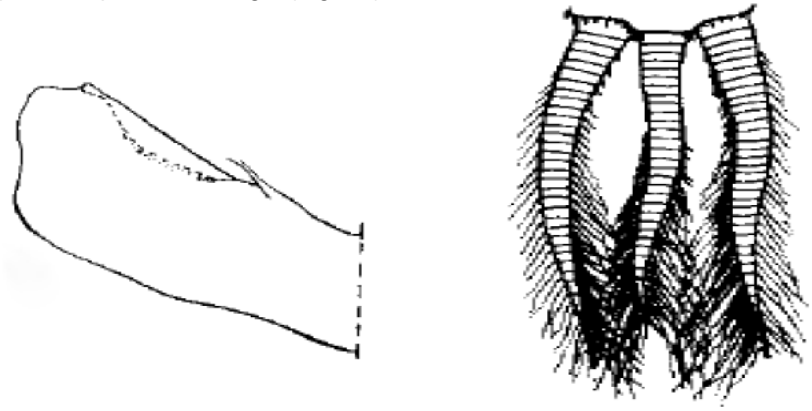


Fig. 34.
(after Müller-Liebenau, [30])

- Hindwing pads minute but visible……**iii**

iii. Segment II of labial palpus dorsally with a row of 4 long, simple setae on distomedial area (Fig. 35b), dorsal margin of femora with 11–15 long (Fig. 35a), clavate setae……..……..***Centroptella ceylonensis***

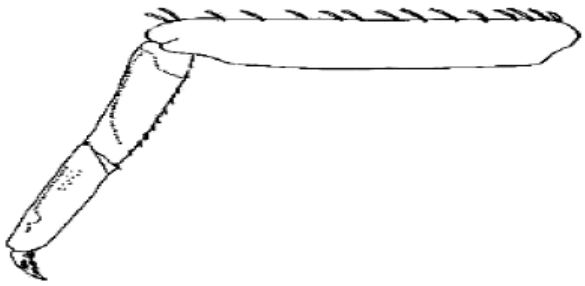




Fig. 35a. **Fig. 35b.**
(after Müller-Liebenau, [30])

- A row of 3 long, simple setae on distomedial area in segment II of labial palpus dorsally, 8–10 long, clavate setae on dorsal margin of femora (Fig. 36)…… ***Centroptella pusilla***

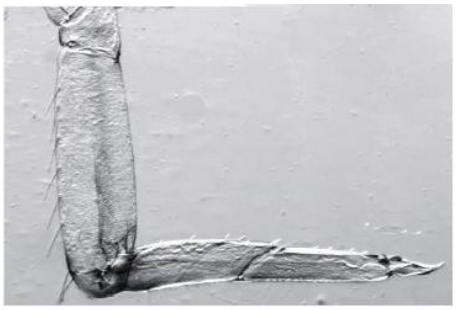


Fig. 36.
(after Selvakumar et al. [31])

iii. Outer incisors of mandibles with a small lateral denticle (Fig. 36b), segment III of labial palpus rounded with distal margin slightly expanded (Fig. 36a)……..***Centroptella soldani***



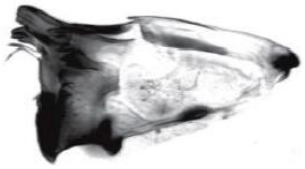
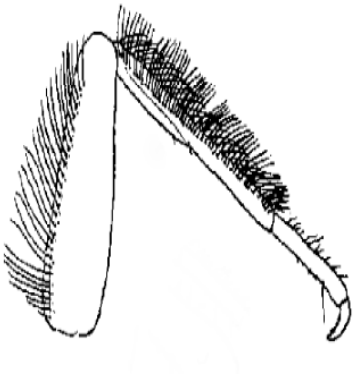


Fig. 36a. **Fig. 36b.**
(after Selvakumar et al. [31])

3. Presence a regular setal row on larval tibia (Fig. 37a)……..***Acentrella***…….**a**
   a. Larval terga lack median hooks; they can be either smooth, or with small tubercles. ……subgenus…….***Liebebiella***……...**i**
      i. Labrum is widest at the base (Fig. 37b)……..***Acentrella vera***



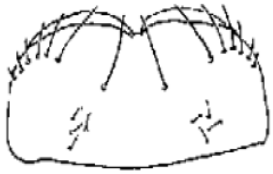
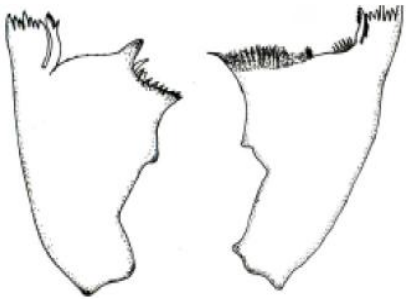


Fig. 37a. **Fig. 37b.**

(after Muller-Liebenau, [32])

4. Segment 2 of maxillary palp without concavity or constrictions……....***Nigrobaetis***……**i**
   i. Presence of hindwing pads; paraproct (Fig. 38b) with a two large and three small spines on distal margin; segment 3 of labial palp truncated or slightly concave; margin between prostheca and mola of both mandibles with reduced number of spines (Fig. 38a); contrasted pattern of the abdominal tergites……....***Nigrobaetis paramakalyani***



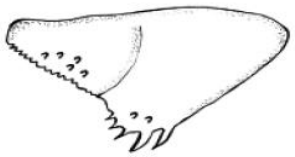


Fig. 38a. **Fig. 38b.**

(after Kubendran et al. [33])

- Segment 2 of maxillary palp distomedially concave or consantennal scapes distolaterally with notch……**5**

5. Labial palpi enlarged, terminal segment of maxillary palpi excavate…….***Labiobaetis***……....**i**

i. Gills in segment I-VII……..……**ii**

- Gills in segment II- VII……..……..**v**

ii. Absence of hind wing pads……...……**iii**

- Well developed hind wing pads……..……..……..**iv**

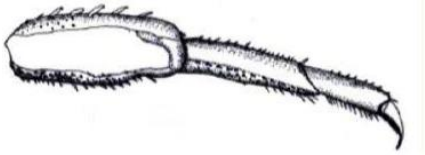
iii. Labrum with a dense submarginal row of clavate bristles (Fig. 39b); frontal margin of 2nd segment of labial palp concave (Fig. 39a)…….....***Labiobaetis germinatus***





Fig. 39a. **Fig. 39b.**
(after Muller-Liebenau & Hubbard, [27])

- Labrum rounded, long and thin setae medially (Fig. 40b); distal margin bordered with setae, distolaterally 14 feathered bristles; presence of 6 pointed setae on the dorsal femoral margin (Fig. 40a)….....***Labiobaetis soldani***



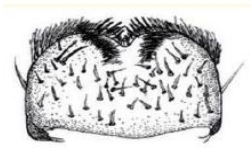


Fig. 40a. **Fig. 40b.**
(after Kubendran et al. [34])

iv. Submarginal bristles on labrum pointed (Fig. 41a), not in a dense row; frontal margin of 2nd segment of labial palp not concave (Fig. 41b)……..……***Labiobaetis pulchellus***





Fig. 41a. **Fig. 41b.**
(after Muller-Liebenau & Hubbard, [27])

iv. Long and stout setae in the dorsal margin of femora (Fig. 42a); spatulate setae on the labrum (Fig. 42b); distomedial projection arched in segment 2 of labial palp (Fig. 42c)……..…….***Labiobaetis jacobusi***



Fig. 42a. **Fig. 42b.** **Fig. 42c**
(after Kubendran et al. [33])

- Labial palpi not enlarged, terminal segment of maxillary palpi not excavate……..……...…..**6**

6. Segment 2 of maxillary palpi without a distomedial modification; antennal scapes without a distolateral notch; median lobe of 2nd segment of labial palp of normal size, not greatly enlarged……***Baetis***……..……**i**

i. Median lobe of 2nd segment of labial palpus greatly enlarged….....**ii**
Median lobe of 2nd segment of labial palpus of normal size (Fig. 43a), not greatly enlarged.....**iii**

ii. Basal segment of antenna without outer apical lobe; indentation at inner apical margin of 2nd segment of maxillary palpus weak to absent; hind wing pads greatly reduced; scale bases on posterior margin of terga rounded (Fig. 43b) .....***Baetis ordinatus***

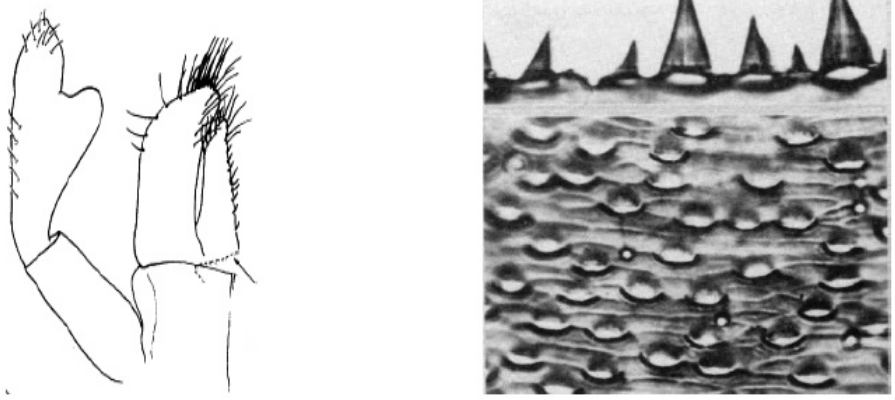


Fig. 43a. **Fig. 43b.**
(after Muller-Liebenau & Hubbard, [27])

iii. 3rd segment of labial palpus rounded at inner margin (Fig. 44b); abdominal gills pigmented above a median longitudinal line, scales and scale bases present only in the pigmented half and along hind margin (Fig. 44a); spines on posterior margin of terga pointed and elongate (Fig. 44c).....***Baetis acceptus***

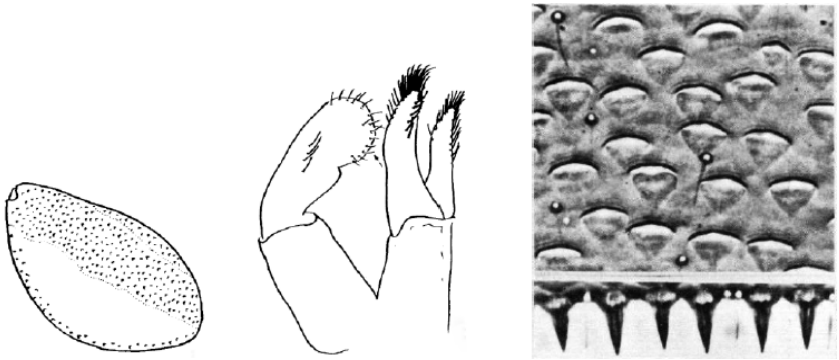


Fig. 44a. Fig. 44b. Fig. 44c
(after Muller-Liebenau & Hubbard, [27])

- 3rd segment of labial palpus rather straight at inner margin (Fig. 45b); no pigmented area on abdominal gills (Fig. 45a), scales and scale bases over entire gill surface; spines on posterior margin of terga pointed, short, rather triangular (Fig. 45c).....***Baetis conservatus***

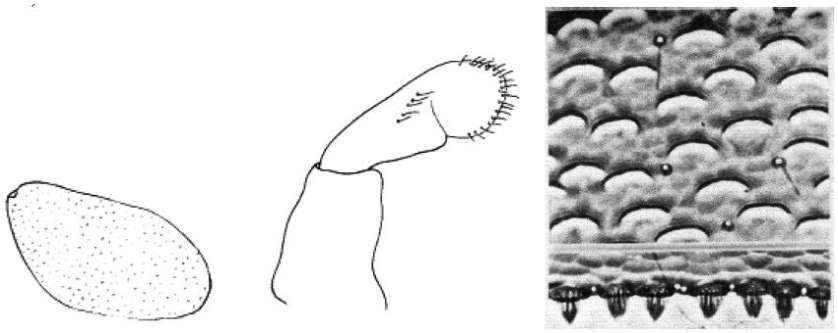


Fig. 45a. Fig. 45b. Fig. 45c
(after Muller-Liebenau & Hubbard, [27])

7. Mandible with smooth medial margin, pointed apex of labial palpus, villopore on femur and paraproct with a patch of notched scales……..…….....***Tenuibaetis***
   i. Posterior half of mesonotum have pale yellow transverse band, presence of paired pale yellow maculae in tergum IV and terga IX–X pale yellowish (Fig. 46a); segment 2 of labial palp have row of 6 long setae (Fig. 46c); gills 1–7 with poorly developed tracheae and with serrated margin (Fig. 46b)……...***Tenuibaetis frequentus***

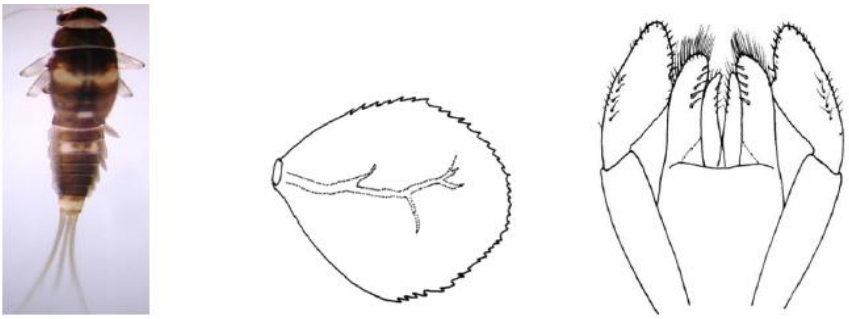


Fig. 46a. Fig. 46b. Fig. 46c.
(after Kubendran et al. [33])

4. CONCLUSION

This work serves as a taxonomic primer for doing further research on mayfly taxonomy, biology, biogeography and ecology and it will also aid Bio monitoring programs in the Palni and Cardamom hills.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Saunders DL, Meeuwing JJ, Vincent ACJ. Freshwater protected areas: Strategies for conservation. Conservation Biology. 2002;16(1):30-41.
2. Moretti MS, Callisto M. Biomonitoring of benthic macroinvertebrates in the middle Doce River watershed. Acta Limnologica Brasiliensia. 2005;17(3):267-281.
3. Resh VH, Rosenberg DM. The ecology of aquatic insects. Praeger Publishers, New York. 1984;625.
4. Merritt RW, Cummins KW. An introduction to the aquatic insects of North America. Dubuque, Kendall/hunt, Iowa. 1984;722.
5. Barathy S, Sivaruban T, Srinivasan P. Distribution of mayflies in thirty streams of Western Ghats, Southern India. Journal of Insect Biodiversity. 2020;18(2):50-62.
6. Sivaramakrishnan KG, Madhyastha NA, Subramanian KA. Field guide to aquatic macroinvertebrates. Life Scape IISc, Bangalore. 1998;8.
7. Balasubramanian C, Venkataraman K, Sivaramakrishnan KG. Bioecological studies on the burrowing mayfly *Ephemera nadinae* McCafferty and Edmunds 1973 (Ephemeroptera: Ephemeridae) in Kurangani Stream, Western Ghats. Journal of the Bombay Natural History Society. 1993;89:72-77.
8. Selvakumar C, Sivaramakrishnan KG, Janarthanan S. A new record of *Potamanthellus caenoides* Ulmer 1939 (Ephemeroptera: Neoephemeridae) from the Southern Western Ghats of India. Biodiversity Data Journal. 2015;3:e5021.
   DOI: https://doi.org/10.3897/BDJ.3.e5021
9. Soldán T. Two new species of *Clypeocaenis* (Ephemeroptera: Caenidae) with a description of adult stage and biology of the genus. Acta Entomologica Bohemoslovaca. 1983;80(3):195–206.
10. Sivaramakrishnan KG, Peters WL. Description of a new species of *Notophlebia* from India and reassignment of the ascribed nymph of *Nathanella* (Ephemeroptera: Leptophlebiidae). Aquatic Insects. 1984;6(2):115–121.
11. Grant PM, Sivaramakrishnan KG. A new species of *Thraulus* (Ephemeroptera: Leptophlebiidae) from Southern India. Florida Entomologist. 1985;68(3):424–432.
12. Sivaramakrishnan KG. A new genus and species of Leptophlebiidae: Atalophlebiinae from southern India (Ephemeroptera). International Journal of Entomology. 1984;26(3):194–203.
13. Sivaramakrishnan KG, Venkataraman K, Balasubramanian C. Biosystematics of the genus *Nathanella* Demoulin (Ephemeroptera:Leptophlebiidae:Atalophlebiinae) from Southern India. Aquatic Insects. 1996;18(10):19–28.
14. Rekha K, Anbalagan S, Dinakaran S, Balachandran C, Krishnan M. A new mayfly species of *Choroterpes* (*Euthraulus*) (Ephemeroptera: Leptophlebiidae) from South India. Zootaxa. 2019;4565(4):539–544.
   DOI: http://dx.doi.org/10.11646/zootaxa.4565.4.8
15. Tong X, Dudgeon D. *Choroterpes* (*Choroterpes*) *petersi*, a new species of Leptophlebiidae (Insecta: Ephemeroptera) from China. Pan-Pacific Entomologist. 2003;79(1):71–74.
16. Sivaramakrishnan KG. New genus and species of Atalophlebiinae (Ephemeroptera: Leptophlebiidae) from Southern India. Annals Entomological Society of America. 1985;78:235–239.

17. Peters WL, Edmunds GF Jr. Revision of the generic classification of Eastern Hemisphere Leptophlebiidae (Ephemeroptera). Transactions of the Royal Entomological Society of London. 1970;116:225–253.
18. Selvakumar C, Sivaruban T, Subramanian KA, Sivaramakrishnan KG. A new genus and species of Atalophlebiinae (Insecta: Ephemeroptera: Leptophlebiidae) from Palni hills of the Southern Western Ghats, India. Zootaxa. 2016;4208(4):381–391.
DOI: http://doi.org/10.11646/zootaxa.4208.4.5
19. Selvakumar C, Sivaramakrishnan KG, Janarthanan S, Arumugam M, Arunachalam M. Impact of riparian land-use patterns on Ephemeroptera community structure in river basins of the Southern Western Ghats, India. Knowledge and Management of Aquatic Ecosystems. 2014;412:11.
DOI: https://doi.org/10.1051/kmae/2013093
20. Selvakumar C, Sivaramakrishnan KG, Jacobus LM, Janarthanan S, Arumugam M. Two new genera and five new species of Teloganodidae (Ephemeroptera) from South India. Zootaxa. 2014;3846(1):87–104.
21. Sartori M, Peters JG, Hubbard MD. A revision of Oriental Teloganodidae (Insecta, Ephemeroptera, Ephemerelloidea). Zootaxa. 2008;1957:1–51.
22. Wang TQ, McCafferty WP. New genus of Teloganodinae (Ephemeroptera: Pannota: Ephemerellidae) from Sri Lanka. Bulletin de la Société d'Histoire Naturelle de Toulouse. 1996;132:15–18.
23. Anbalagan S, Balachandran C, Kannan M, Dinakaran S, Krishnan M. First record and a new species description of *Dudgeodes* (Ephemeroptera: Teloganodidae) from South India. Turkish Journal of Zoology. 2015;39:308–313.
24. Sivaruban T, Barathy S, Arunachalam M, Venkataraman K, Sivaramakrishnan KG. *Epeorus petersi*, a new species of Heptageniidae (Ephemeroptera) from the Western Ghats of Southern India. Zootaxa. 2013;3731(3):391–394.
25. Venkataraman K, Sivaramakrishnan KG. A new species of *Cinygmina* (Ephemeroptera: Heptageniidae) from South India and reevaluation of generic traits of *Cinygmina* Kimmins 1937. Hexapoda. 1989;1(1–2):117–121.
26. Venkataraman K, Sivaramakrishnan KG. A new species of *Thalerosphyrus* from South India (Ephemeroptera: Heptageniidae). Current Science. 1987;56(21):1126-1129.
27. Müller-Liebenau I, Hubbard MD. Baetidae from Sri Lanka with some general remarks on the Baetidae of the Oriental region (Insecta, Ephemeroptera). The Florida Entomologist. 1985;68:537–561.
28. Selvakumar C, Sundar S, Sivaramakrishnan KG. Two new mayfly species (Baetidae) from India. Oriental Insects. 2012;45(2):116-129.
DOI: http://dx.doi.org/10.1080/00305316.2012.689487
29. Kluge NJ, Godunko RJ, Svitok M. Nomenclatural changes in *Centroptella* Braasch & Soldán, 1980 (Ephemeroptera, Baetidae). ZooKeys. 2020;914:81.
30. Müller-Liebenau I. Three new species of the genus *Centroptella* Braasch & Soldán, 1980, from Sri Lanka (Insecta:Ephemeroptera). Archiv für Hydrobiologie. 1983;97:486–500.
31. Selvakumar C, Kubendran T, Kailash Chandra, Avtar Kaur Sidhu. First record of the genus *Bungona* (Harker 1957) (Ephemeroptera: Baetidae) and range extension of two species belonging to *Bungona* in India. Journal of Entomological Research. 2017;41(4):373-376.
32. Muller-Liebenau I. Five new species of *Pseudocloeon* Klapalek, 1905, (Fam. Baetidae) from the Oriental Region (Insecta, Ephemeroptera) with some general remarks on *Pseudocloeon*. Archiv fur Hydrobiologie. 1982;95(14):283-295.
33. Kubendran T, Balasubramanian C, Selvakumar C, Gattolliat JL, Sivaramakrishnan KG. Contribution to the knowledge of *Tenuibaetis* Kang and Yang 1994, *Nigrobaetis* Novikova and Kluge 1987 and *Labiobaetis* Novikova and Kluge 1987 (Ephemeroptera: Baetidae) from the Western Ghats (India). Zootaxa. 2015;3957(2):188–200.
34. Kubendran T, Rathinakumar T, Balasubramanian C, Selvakumar C, Sivaramakrishnan KG. A new species of *Labiobaetis* Novikova and Kluge 1987 (Ephemeroptera: Baetidae) from Southern Western Ghats of India, with comments on the taxonomic status of Labiobaetis. Journal of Insect Science. 2014;14(86):1-10.

Biography of author(s)



Dr. S. Barathy, Assistant Professor
Department of Zoology, Fatima College, Madurai-625018, India.

She is an Assistant Professor at the Department of Zoology, Fatima College, Madurai-625018, India. Her Research is mainly focused on aquatic entomology and diversity studies. She published around 21 research articles in reputed National and International Journals. She presented papers in various colleges and Universities. She has more than ten years of teaching experience. To make entomological research work more perfect the light of research is extended to Molecular taxonomy. Three new species is identified and published in a reputed journal and the same type of work is been continued.



Dr. T. Sivaruban, Assistant Professor
PG& Research Department of Zoology, The American College, Madurai-625002, India.

He is an Assistant Professor at PG& Research Department of Zoology, The American College, Madurai-625002, India. He has completed Doctorate degree from Manonmanium Sundaranar university, South India. His field of interest is aquatic Entomology and Molecular taxonomy. Teaching is his passion and has 17 years of teaching experience. He has published more than 24 research articles in National and International peer review Journals. He has recorded four new species of Mayfly from western Ghats. He is extending his research work on finding of new species, Taxonomy and diversity of Ephemeroptera, Trichoptera and plecoptera from western and Eastern Ghats of India.



Pandiarajan Srinivasan
PG& Research Department of Zoology, The American College, Madurai-625002, India.

He is currently pursuing his Ph.D in The American College, Madurai affiliated to Madurai Kamar University. His core areas of research are taxonomy, ecology and molecular systematics of mayflies more especially Pannota mayflies. He has published 12 research articles in national and international journals. He has recorded two new mayfly species (*Caenis* and *Sparsorythus*) from Western Ghats of Southern India.

© Copyright (2021): Author(s). The licensee is the publisher (Book Publisher International).

Chapter 11
Print ISBN: 978-93-90516-93-3, eBook ISBN: 978-93-90516-94-0

HMB Supplementation and Resistance Training: Current Overview on Inflammation, Oxidative Stress and Cardiovascular Risk Factors

Hamid Arazi[1*], Behzad Taati[1] and Katsuhiko Suzuki[2]

DOI: 10.9734/bpi/rrab/v5/7486D

ABSTRACT

β-hydroxy β-methylbutyrate (HMB), a bioactive metabolite of the branched-chain amino acid leucine, is a popular dietary supplement among different athletes who participate in regular resistance training for muscle gain and strength. However, HMB has received less attention concerning its impact on changes in inflammation, oxidative stress (OS) and cardiovascular risk factors following resistance training. Therefore, in this chapter we aim to summarize the current literature in this area in a reasoned manner. Due to the small number of published studies, the interpretation of outcomes should be taken cautiously. However, the data reviewed here suggest that acute HMB supplementation may attenuate the pro-inflammatory response following high-intensity resistance exercise in athletes. Moreover, the available findings collectively indicate that chronic HMB consumption and concurrent resistance training does not improve cardiovascular parameters and OS markers greater than resistance training alone. Taken together, there is clearly a need for further well-designed, long-term studies to support these findings and determine whether HMB supplementation affects the adaptations induced by resistance training associated with the body's inflammatory condition, cardiovascular health and antioxidative defense system in humans.

Keywords: Leucine; β-Hydroxy-β-methylbutyrate; branched-chain amino acid; strength training; sports nutrition; inflammatory markers.

1. INTRODUCTION

Some athletes believe that most normal diets do not provide sufficient demands for an optimum performance during intensive training and competitions. Dietary supplements are a commonly used strategy to improve exercise performance and recovery, and many athletes use them as a part of their regular training or competition routine [1]. Over the last decades, numerous studies have been conducted to identify anabolic nutrients for skeletal muscles.

β-hydroxy β-methylbutyrate (HMB) is a type of amino acid supplement on the market. Due to its positive effects on muscle function and protein turnover [2-4], HMB is fast becoming popular among different athletes as an ergogenic aid [5,6]. Indeed, HMB is added to many training protocols, with the hopes of an enhanced lean body mass and sports performance [7]. Scientific research during the past 20 years has demonstrated that HMB supplementation together with resistance training can improve body composition [8-13], muscle strength [2,8,10-14] and power [8,11,14]. It has also been reported that supplying HMB promotes favorable changes in aerobic [15-17] and anaerobic [12,15,18] capacity, and muscle recovery after exercise [13,19-22] in different athletes. To date, several systematic reviews and meta-analyses have investigated the aforementioned actions of HMB supplementation in a variety of populations [5,6,23-27]. Although recent studies have proposed some other actions of HMB with regard to the effects on inflammatory, cardiovascular and oxidative stress (OS) markers,

[1]*Department of Exercise Physiology, Faculty of Sport Sciences, University of Guilan, Rasht 4199843653, Iran.*
[2]*Faculty of Sport Sciences, Waseda University, Tokorozawa 359-1192, Japan.*
**Corresponding author: E-mail: hamidarazi@yahoo.com;*

these new aspects have received less attention. Therefore, we aimed to review recent findings in these contexts.

2. A BRIEF OVERVIEW OF HMB METABOLISM

HMB is a metabolic by-product of the essential branched-chain amino acid (BCAA), leucine, which has key roles in protein metabolism [28]. Fig. 1 shows the different steps in the production of HMB [26,29]. Following the reversible transamination of leucine to α-ketoisocaproate (KIC) through the enzymatic action of BCAA aminotransferase [27], KIC in the liver can either produce isovaleryl-CoA by the enzyme, branched-chain α-ketoacid dehydrogenase [29], or generate HMB by the cytosolic enzyme, KIC dioxygenase [30]. Most amounts of produced KIC are metabolized into isovaleryl-CoA, and it has been estimated that only approximately 2–10% of leucine is oxidized into HMB [26,31]. The normal plasma range of HMB concentrations is 1 to 4 μmol/L, but can increase 5-to 10-fold following leucine administration [32]. Although some foods, including citrus fruits, some fish, and breast milk, have some HMB [29], it is impractical to provide the typical 3 g daily dosage of HMB used in most previous human studies that demonstrate an improvement of body composition [8,9,11-13] and muscle strength [2,8,11-14]. Therefore, HMB supplementation is a reasonable way for different athletes, specifically, those who participate in resistance training programs.

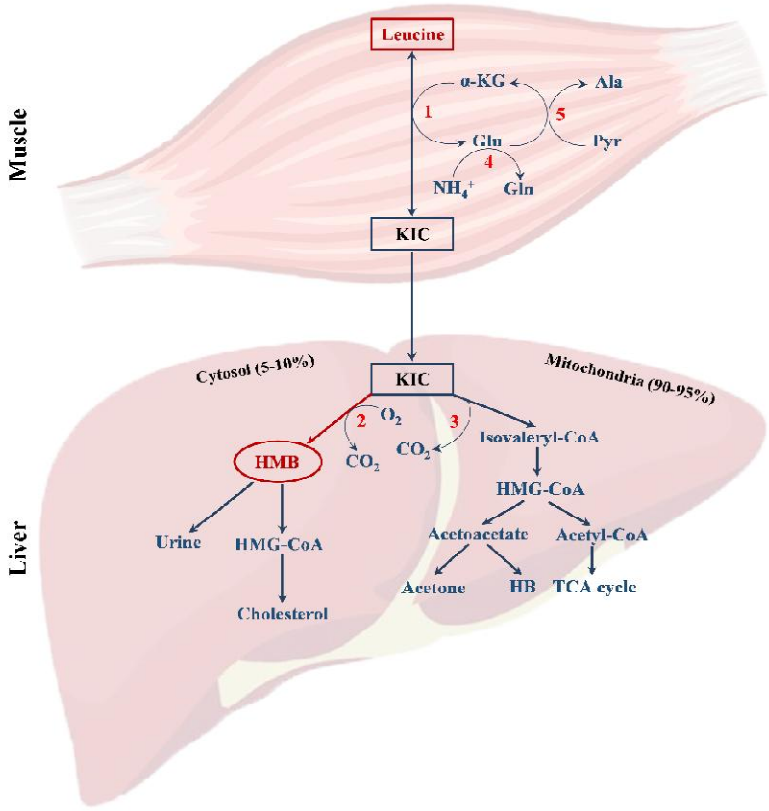


Fig. 1. Pathways of Beta-hydroxy-beta-methylbutyrate (HMB) metabolism from the amino acid leucine. Modified from Nissen and Abumrad [32]

KIC: Alpha-ketoisocaproic acid, HB: Beta-hydroxybutyrate, HMG-CoA: β-Hydroxy β-methylglutaryl-coenzyme A, Ala: Alanine, Pyr: Pyruvate, Glu: glutamate, Gln: glutamine, TCA: tricarboxylic acid, 1: branched-chain amino acid (BCAA) aminotransferase, 2: KIC dioxygenase, 3: branched-chain alpha-keto acid dehydrogenase (BCKAD), 4: Glutamine synthetase, 5: Alanine aminotransferase

There are two commercially available forms of HMB supplement, including (i) calcium HMB (HMB-Ca), a mono-hydrated calcium salt; and (ii) a free acid form of HMB (HMB-FA) [24,33]. Depending upon the dose and its ingestion with other additional nutrients, the magnitude and rate of appearance

in blood circulation, and the clearance rate of HMB following consumption are different. In this context, Vukovich et al. [34] compared two doses of HMB-Ca and found that a 3-g dose can cause a peak in plasma concentrations of HMB 1 h after consumption, while a peak HMB level occurred 2 h after the ingestion of a 1-g dose. Plasma concentrations of HMB and urinary losses with the 3-g dose were also significantly higher than the 1-g dose (300% and 14%, respectively). The authors also reported that adding 75 g of glucose to the HMB-Ca dosage may delay peak HMB concentrations by 1 h and decrease its magnitude because of slow gastric emptying or an improvement in HMB clearance [34]. However, compared to 1 g of HMB-Ca, the absorption rate of 0.8 g of HMB-FA was higher and it only took 36 min to reach peak plasma concentrations following ingestion [33]. Furthermore, these increases in peak plasma levels were accompanied by a higher plasma clearance rate (25%) compared to HMB-Ca, indicating greater tissue uptake and utilization [24,26,35]. Although these benefits provided more effective and practical effects on muscle recovery after exercise through the greater intra-muscular HMB bioavailability [24,33,36], another study reported higher bioavailability of HMB after HMB-Ca intake when compared with an equivalent dosage of HMB-FA [37]. Taken together, most published studies have administered HMB-Ca, and further research is needed in this area.

3. AN OVERVIEW OF DIFFERENT HMB EFFECTS AND ITS POTENTIAL MECHANISMS

We have summarized a number of the most important and beneficial effects of HMB supplementation and its suggested mechanisms of action (Table 1).

Table 1. A summary of potential mechanisms proposed for the different beneficial effects of HMB supplementation on skeletal muscle

Effects	Mechanisms of action
Increasing protein synthesis	• Stimulation of the mTOR signalling pathway: Inhibition of MuRF-1 expression and phosphorylation of FoxO1 and FoxO3a through activation of the PI3K/Akt signalling pathway [3]; increasing the expression of MyoD and MEF2, activation of the MAPK/ERK and PI3K/Akt pathways, leading to myogenic cell proliferation [38]. • Stimulation of the GH/IGF-1 axis: Increasing the mRNA expression of pituitary GH and serum concentrations of IGF-1 [39].
Decreasing proteolysis (protein breakdown)	• Down-regulation of catabolic signalling pathways, including ubiquitin-proteasome and autophagy-lysosome systems: Inhibiting proteasome expression, reducing activities of proteasome enzyme, down-regulation of caspases, decreasing the apoptosis of myonuclei [40-43]. • Increasing GH and IGF-1 [44].
Enhancing tissue repair	• Increasing proliferation of satellite cells [38]. • Increasing cholesterol synthesis: HMB acts as a substantial precursor of cell membranes and improves the repair of sarcolemma after contractile activity [45].
Improving excitation-contraction coupling in muscle cells	• Increasing calcium release from the sarcoplasmic reticulum [46].
Improving aerobic capacity	• Increasing mitochondrial biogenesis and fat oxidation [47]. Increasing the HSL gene and protein expression in white adipose tissue because of increased GH levels, leading to increased lipolysis, and thus, more lipid availability [29,39].
Delaying acute muscle fatigue	• Increasing the content of mitochondrial acetyl-CoA through the conversion of HMG-CoA into acetoacetyl-CoA [31,32].
Increasing ATP and glycogen content in skeletal muscle	• Accelerating the TCA cycle, increasing malate-aspartate shuttle, and providing needed carbon for glycogen synthesis [48].

HMB: β-hydroxy β-methylbutyrate, mTOR: mammalian target of rapamycin, MuRF-1: muscle RING-finger protein-1, FoxO: forkhead box protein class O, PI3K/Akt: phosphatidylinositol 3-kinase/protein kinase B, MyoD: myoblast determination protein 1, MEF2: myocyte enhancer factor-2, MAPK/ERK: mitogen-activated protein kinase/extracellular-signal-regulated kinase, GH: growth hormone, IGF-1: insulin-like growth factor-1, HSL: hormone-sensitive lipase, HMG-CoA: β-Hydroxy β-methylglutaryl-coenzyme A, ATP: adenosine triphosphate, TCA: tricarboxylic acid

4. EFFECTS OF HMB ON INFLAMMATION

4.1 Effects on Inflammation without Exercise

HMB improves immune function, especially under stressful conditions. *In vitro*, HMB has been shown to increase lymphocyte blastogenesis in a dose-dependent fashion [32]. In an animal study, Peterson et al. [49] found that HMB enhanced nitrite production in macrophages and also antibody production. The favorable effects of HMB supplementation on the number of CD3 and CD8 cells and human immunodeficiency virus (HIV) load have also been reported [50]. These data were supported by Hsieh et al. [51], who concluded that supplementation of HMB at 3 g/day for seven days may have an anti-inflammatory effect in a group of elderly patients with chronic obstructive pulmonary disease (COPD).

4.2 Effects on Inflammation Following Exercise

Today, exercise training is considered as a valuable non-pharmacological therapy in many international societies which has led to the statement of “Exercise is Medicine” [52]. However, it has been well documented that acute exercise, specifically high-intensity exercise, results in increased inflammatory markers [53]. In contrast, regular exercise training exerts anti-inflammatory effects on the blood [54]. The investigation of new strategies for decreasing the body’s inflammatory reactions after exercise may provide further insight into improved recovery and subsequent performance. It has been suggested that some pro-inflammatory factors, such as interleukin (IL) 1 beta (IL-1β) and tumor necrosis factor alpha (TNF-α), increase proteolysis and may modulate protein turnover [55]. Because HMB is associated with less proteolysis [40,42], to date, several studies have focused on unraveling whether HMB supplementation affects the inflammatory responses following exercise training (Table 2). Beneficial effects of acute HMB supplementation on attenuating pro-inflammatory mediators have been reported in resistance-trained athletes [56,57]. Townsend et al. [56] showed that circulating pro-inflammatory markers, TNF-α and monocyte TNF-α receptor 1 (TNFR1) expression were elevated during an acute bout of heavy resistance exercise and subsequent recovery in healthy, resistance-trained men. However, HMB-FA supplementation (acute ingestion before and/or after exercise) decreased these mediators immediately after resistance exercise. On the other hand, another study did not support these findings. Additionally, in another well-designed study, acute HMB ingestion (30 min before, and 2 and 6 h after exercise) had no effect on the responses of macrophage inflammatory protein (MIP)-1β, but attenuated the significant peak expression of complement receptor type 3 (CR3) at 30 min post-exercise (four sets of up to 10 repetitions of three resistance exercises at 70–80% 1-repetition maximum). No increases in CR3 expression after exercise or during recovery was also observed by HMB. However, these changes did not contribute to a more rapid recovery or improve subsequent performance [57]. Longer duration of HMB supplementation may also have some beneficial effects on the inflammatory response after exercise. Hoffman et al. [58] revealed that 23 days of HMB supplementation in combat soldiers can attenuate the inflammatory response (TNF-α, IL-8, IL-10, granulocyte colony-stimulating factor, interferon-γ, and fractalkine) to intense military training, and maintain muscle quality.

Although these data provide evidence for a potential blunted or delayed inflammatory response following intense exercise protocols with acute or three weeks of HMB supplementation, anti-inflammatory effects of HMB metabolites were not confirmed by others that examined acute or long-term effects of HMB. A recent study has demonstrated that daily consumption of different leucine metabolites including α-hydroxyisocaproic acid (α-HICA), HMB-FA, and HMB-Ca has no effect on fasting plasma IL-6 and TNF-α concentrations in resistant-trained men, measured at the end of weeks 4 and 8 of a whole-body resistance training protocol [59]. On the acute basis, Vulcan [60] investigated the effect of HMB ingestion (before, or before and after exercise) on inflammatory responses following three sets of 50 eccentric leg extensions on each leg. It was observed that a decrease in serum concentration of IL-1 receptor antagonist (IL-1ra) and TNF-α for the placebo group was attenuated by HMB at 48-h and 72-h post exercise. In another study, it was investigated whether a longer period of HMB administration can influence inflammatory mediators in elite, national team level adolescent volleyball players. In this study, Portal et al. [18] found that 7-week consumption of HMB (3 g/day) did not change serum concentrations of IL-6 and IL-1ra during the early phase of the volleyball season. It should be considered that the training stimulus in this research was different from that performed by the four aforementioned studies [56-58,60]. Therefore, additional studies with long-term

supplementation with HMB (at least 10 to 12 weeks) are needed to determine the impact of this dietary supplement on inflammatory responses to resistance exercise.

Because of limited scientific data regarding HMB effects on OS and cardiovascular parameters following resistance exercise, we propose new approaches for future research.

5. AN APPROACH TO OXIDATIVE STRESS

To the best of our knowledge, the available scientific literature on the effect of HMB supplementation on OS in humans is still preliminary in nature and it should be taken into more accurate consideration. Although HMB may improve immune function in humans [56,57,60], unfortunately, there are only two studies [61,62] that has directly examined the effects of HMB supplementation on OS responses to exercise training (Table 2). In a randomized, double-blind, placebo-controlled trial, our research team investigated the effects of six-week HMB-FA supplementation on OS markers in 16 healthy young males. In this study, 8-hydroxy-2-deoxyguanosine (8-OHdG), malondialdehyde (MDA), and protein carbonyl (PC) were measured 48 h before and after resistance training. A significant decrease in MDA and PC was observed in both placebo and HMB groups. However, 8-OHdG did not change after resistance training in any of the groups. Thus, it seems that adding HMB supplementation to resistance training had no further improvements related to OS markers [61]. In contrast, consumption of 1 g of HMB-FA 30 min prior to performing an acute plyometric exercise protocol, including 15 sets of 10 repetitions of maximal-effort vertical jumps, attenuated OS responses to exercise compared with placebo. In this study, we observed that physically active males who had consumed HMB-FA before the exercise had lower serum levels of 8-OHdG, MDA, and PC at 1 h post-exercise [62]. A transient increase in OS markers following acute exercise may be linked to the health benefits of regular exercise. In fact, long-term exercise training elicits a variety of positive health effects on immune system which is thought to be associated with the adaptations induced by acute exercise sessions over time. Moreover, these results provide initial support for the efficacy of HMB-FA supplementation in promoting recovery from OS responses induced by plyometric-type eccentric exercise. It is not clear what suppression of this oxidative response may do long-term with HMB supplementation. Given the limited available data about HMB effects on OS mediators, more research examining its effects is warranted. Only after more well-designed trials of HMB have been performed and its effects on the OS profile have been better defined will it be possible to comment on the effectiveness of HMB as a dietary supplement.

6. AN APPROACH TO CARDIOVASCULAR RISK FACTORS

6.1 Cardiovascular Effects of HMB without Exercise

As shown in Fig. 1, cytosolic β-hydroxy-β-methylglutarate-Co-A (HMG-CoA), which is produced from HMB in the cytosol of the liver, can be used for cholesterol synthesis [63]. Thus, HMB action as a precursor for cellular cholesterol synthesis can be important for membrane production during periods of high muscular stress, especially during exercise training and the subsequent recovery period. This is known as the cholesterol synthesis hypothesis (CSH) [64]. There are controversial findings about the effect of HMB on some cardiovascular risk factors. For example, different authors have found no change [65], an increase [66], or a decrease [64] in low-density lipoprotein (LDL) or total cholesterol (TC). In a comprehensive study, Nissen et al. [64] summarized data from nine studies in which humans were fed 3 g HMB/day for three to eight weeks and reported that HMB supplementation results in a net decrease in TC (5.8%), a decrease in LDL cholesterol (7.3%), and a decrease in systolic blood pressure (4.4 mmHg). However, Hsieh et al. [65] concluded that HMB supplementation (2 g/day) for two or four weeks has no significant effect on serum lipids in bed-ridden elderly men and women. More recently, an animal study examined the effectiveness of a 12-week HMB administration on insulin resistance induced by a high fructose diet in rats. Compared to the control group, HMB significantly enhanced insulin tolerance and decreased fasting insulin, insulin resistance index (HOMA-IR), glycosylated hemoglobin (Hb_{A1c}), hepatic glycogen content, and serum triglycerides (TG), LDL, and very low-density lipoprotein (VLDL) [67]. It has also been demonstrated that two-week supplementation with HMB reduces arrhythmias during ischemia induced in rats [68]. Generally, these data suggest that HMB supplementation may result in a decrease in the risk of heart attack and stroke.

Table 2. Main characteristics of studies examining the effects of HMB supplementation on inflammatory, cardiovascular, and oxidative stress markers

Author (Year)	Subjects	Resistance exercise protocol	Supplementation	Primary findings	Conclusion
Teixeira (2019) [59]	40 healthy men	8 weeks; 3 sessions/week; 3-4 sets of barbell back squat, deadlift, machine leg extension, barbell flat bench press, dumbbell military press, lat pull-down, seated cable row with 8-12 RM; 60-120 s rest intervals	α-HICA: 3 × 500 mg/day HMB-FA and -Ca: 3 × 1 g/day Placebo: magnesium stearate	↔ IL-6, hs-CRP, and TNF-α at the end of weeks 4 and 8	No leucine metabolite attenuated inflammatory markers during training
Townsend (2013) [56]	40 resistance-trained men	4 sets of the squat (80% of 1RM), and dead lift and barbell split-squat (70% of 1RM); as many repetitions as possible (up to 10 repetitions for each set); 90 s rest intervals between sets and exercises	3 g (1-g serving of HMB-FA, 30 min before, and 2 h and 6 h following the exercise session)	↑ TNF-α from pre to immediately post-exercise in only PL group; ↑ TNFR1 expression from pre to 30 min post-exercise in only PL group	Acute HMB-FA supplementation may attenuate the initial immune responses to an intense RE, which may reduce subsequent recovery period
Vulcan (2012) [60]	Untrained subjects (16 men, 16 women)	3 sets of 50 eccentric leg extensions from 0° to 90° at a rate of 60°/sec on both legs; 2 min rest intervals between sets	Pre-exercise, or pre-exercise and for 4 days (3 servings/day) post-exercise; either HMB-Ca or HMB-FA	↑ IL-1ra at 48 h, 72 h, and 96 h post-exercise compared to PL; ↑ TNF-α at 48 h, and 72 h post-exercise compared to PL	The role of acute HMB supplementation on reducing the inflammatory response after RE has not been confirmed
Gonzalez (2014) [57]	39 resistance-trained men	4 sets of the squat, dead lift and barbell split-squat (70–80% of 1RM); as many repetitions as possible (not to exceed 10 repetitions in any set); 90 s rest intervals between sets and exercises	3 g (1-g serving of HMB-FA, 30 min before, and 2 h and 6 h following the exercise session)	↔ MIP-1β responses; ↓ peak expression of CR3 at 30 min post-exercise; ↑ percentage of monocytes expressing CR3 for up to 48 h post-exercise	HMB supplementation may alter immune cell mobilization and adhesion mechanisms during tissue recovery after RE
Hoffman (2016) [58]	11 elite combat male soldiers	A combination of different military training including combat skill development, extreme trainings, navigational training with carrying approximately 35 kg of equipment	23 days HMB-FA: 3 × 1 g/day	↓ TNF-α responses; ↓ G-CSF; ↓ IL-10; ↓ INFγ; ↓ IL-8; ↓ CX3CL1 from pre- to post-training	HMB supplementation may attenuate the inflammatory mediators to severe military trainings, and maintain muscle quality

Author (Year)	Subjects	Resistance exercise protocol	Supplementation	Primary findings	Conclusion
Portal (2011) [18]	Adolescent elite volleyball players (14 males, 14 females)	Volleyball training (the early phase of the volleyball season)	3 g/day HMB supplementation for 7 weeks; the type of HMB has not been mentioned	↔ IL-10 ↔ IL-1ra from pre- to post-training	HMB supplementation has no significant effect on the inflammatory mediator changes during the initial phases of volleyball training season
Arazi (2018) [61]	16 healthy young males	6 weeks; 2 sessions/week; 3 sets of 8–12 repetitions with 75–85% of 1RM (leg press, knee extension, knee flexion, lat pull-down, bench press, shoulder press, cable biceps curl and triceps push down); 2 and 3 min rest intervals between sets and exercises, respectively	HMB-FA: 3 × 1 g/day	↓ MDA and PC from pre- to post-training in both HMB and PL groups; ↔ 8-OHdG in the groups	HMB supplementation has no further improvements related to OS markers in young males
Arazi (2015) [70]	20 male athletes (without regular resistance training)	2 sets of 9 exercises including squat, knee extension, knee flexion, leg press, bench press, lat pull- down, shoulder press, cable biceps curl, and triceps push down; 10 repetitions with 80–85% of 1RM; 2 and 3 min rest intervals between sets and exercises, respectively	HMB-Ca: 3 × 1 g/day	↓ TC, LDL, and TG from pre- to post-training in both HMB and PL groups; ↓ systolic BP and ↑ HDL in HMB group; ↔ diastolic BP, RBC, Hb, Hct, MCV, and MCH in the groups	HMB supplementation is safe and does not result in any adverse effects on cardiovascular parameters in male athletes
Aghaei (2019) [69]	adult male Sprague-Dawley rats	2 weeks; 5 days/week; eccentric resistance training including stepping up from ladder until exhaustion; 2 min rest intervals between sets	450 mg/kg body weight/day; the type of HMB has not been mentioned	↓ hs-CRP, TC, TG, LDL in training-HMB group compared with the control condition	combination of HMB and resistance training may help to improve inflammatory statuses and blood lipid profile

↑ increase, ↓ decrease, ↔ no change, HMB: β-hydroxy β-methylbutyrate, Ca: calcium, FA: free acid, α-HICA: α-hydroxyisocaproic acid, OS: oxidative stress, PL: Placebo, RE: Resistance Exercise, 1RM: 1-repetition maximum, IL-1ra: IL-1 receptor antagonist, MIP: Macrophage inflammatory protein, CR3: Complement receptor type 3, G- CSF: Granulocyte colony-stimulating factor, INFγ: Interferon-γ, CX3CL1: Fractalkine, 8-OHdG: 8-hydroxy-2-deoxyguanosine, MDA: Malondialdehyde, PC: Protein carbonyl, TC: Total cholesterol, TG: Triglycerides, LDL: Low-density lipoproteins, HDL: High-density lipoproteins, BP: Blood pressure, RBC: Red blood cells, Hb: Hemoglobin, Hct: Hematocrit, MCV: Mean corpuscular volume, MCH: Mean corpuscular hemoglobin.

6.2 Cardiovascular Effects of HMB Following Exercise

There are few studies that address the cardiovascular effects of HMB supplementation in conjunction with exercise training (Table 2). More recently, an animal study has shown that a high dosage of HMB supplementation (450 mg/kg body weight/day) together with resistance training for 2 weeks might induce positive effects on the responses of hs-CRP, TC, TG, and LDL following a session of strenuous eccentric resistance exercise in adult male rats [69]. It is worth mentioning here that it is not clear which form of HMB supplement (FA or Ca) was used in this study. Nevertheless, Teixeira et al. [59] revealed that combination of resistance training and using leucine metabolites (α-HICA, HMB-Ca, and HMB-FA) with a routine dosage of 1.5 to 3 g/day for a longer duration (8 weeks) had no effect on hs-CRP in human subjects. Although other cardiovascular risk factors were not measured in this study, we previously reported for the first time that a 4-week supplementation of HMB-Ca at a dose of 3 g/day and concurrent resistance training (3 sessions/week) did not change blood lipid profile of athletes compared with resistance training alone. However, diastolic blood pressure was reduced only in the HMB group [70]. Because of limited data regarding this research topic, it is difficult to declare the certain and exact cardiovascular effects of HMB supplementation when combined with exercise training. Thus, further prolonged investigations are needed to determine these effects.

7. CONCLUSIONS

The available data collectively indicate that acute ingestion of HMB before and after resistance exercise can attenuate some circulating pro-inflammatory mediators, which improves the subsequent recovery process. However, more research is needed to support these effects and verify if chronic HMB consumption and resistance training has more favorable effects on pro- and anti-inflammatory markers. Although the number of studies examining the interaction effects of HMB and exercise training on inflammation, OS, and cardiovascular parameters are limited, it seems that adding HMB supplements to a resistance exercise protocol did not produce further benefits. Generally, future research should be performed to specify the effectiveness of HMB supplementation on the inflammatory profile, the body's antioxidative defense system and OS markers, and cardiovascular risk factors, when combined with exercise training. It should also be noted that despite being an effective strategy to increase strength and prevent muscle mass loss, more attention is required on the dosage and time of HMB supplementation to avoid unwanted alterations [71]. Nonetheless, exercise is effective to reduce inflammation and chronic diseases, and better combinations with dietary supplementation are warrant for future investigations [72,73].

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Maughan RJ, Depiesse F, Geyer H. The use of dietary supplements by athletes. J Sports Sci. 2007;25(S1):S103-S13.
2. Thomson JS, Watson PE, Rowlands DS. Effects of nine weeks of β-Hydroxy-β-Methylbutyrate supplementation on strength and body composition in resistance trained men. J Strength Cond Res. 2009;23(3):827-35.
3. Kimura K, Cheng XW, Inoue A, Hu L, Koike T, Kuzuya M. β-hydroxy-β-methylbutyrate facilitates pi3k/akt-dependent mammalian target of rapamycin and foxo1/3a phosphorylations and alleviates tumor necrosis factor α/interferon γ–induced murf-1 expression in c2c12 cells. Nutr Res. 2014;34(4):368-74.
4. Shirvani H, Rahmati-Ahmadabad S, Kowsari E, Fry H, Kazemi M, Kaviani M. Effects of 2-week HMB-FA supplementation with or without eccentric resistance exercise on expression of some genes related to muscle protein turnover and serum irisin and IGF-1 concentrations. Gene. 2020;760:145018.
5. Rahimi MH, Mohammadi H, Eshaghi H, Askari G, Miraghajani M. The effects of beta-hydroxy-beta-methylbutyrate supplementation on recovery following exercise-induced muscle damage: A systematic review and meta-analysis. J Am Coll Nutr. 2018;1-10.

6. Sanchez-Martinez J, Santos-Lozano A, Garcia-Hermoso A, Sadarangani K, Cristi-Montero C. Effects of beta-hydroxy-beta-methylbutyrate supplementation on strength and body composition in trained and competitive athletes: A meta-analysis of randomized controlled trials. J Sci Med Sport. 2017;21(7):727-35.
7. Palisin T, Stacy JJ. β-Hydroxy-β-methylbutyrate and its use in athletics. Curr Sports Med Rep. 2005;4(4):220-3.
8. Lowery RP, Joy JM, Rathmacher JA, Baier SM, Fuller JC, Shelley MC, et al. Interaction of beta-hydroxy-beta-methylbutyrate free acid and adenosine triphosphate on muscle mass, strength, and power in resistance trained individuals. J Strength Cond Res. 2016;30(7):1843-54.
9. Stout JR, Fukuda DH, Kendall KL, Smith-Ryan AE, Moon JR, Hoffman JR. β-Hydroxy-β-methylbutyrate (HMB) supplementation and resistance exercise significantly reduce abdominal adiposity in healthy elderly men. Exp Gerontol. 2015;64:33-4.
10. Ferreira H, Gill P, Fernandes Filho J, Fernandes LC. Effects of 12-weeks of supplementation with β-hydroxy-β-methylbutyrate-Ca (HMB-Ca) on athletic performance. J Exerc Physiol Online. 2015;18(2):84-94.
11. Wilson JM, Lowery RP, Joy JM, Andersen J, Wilson SM, Stout JR, et al. The effects of 12 weeks of beta-hydroxy-beta-methylbutyrate free acid supplementation on muscle mass, strength, and power in resistance-trained individuals: A randomized, double-blind, placebo-controlled study. Eur J Appl Physiol. 2014;114(6):1217-27.
12. Dunsmore K, Lowery RP, Duncan N, Davis G, Rathmacher J, Baier S, et al. Effects of 12 weeks of beta-hydroxy-beta-methylbutyrate free acid Gel supplementation on muscle mass, strength, and power in resistance trained individuals (Abstract). J Int Soc Sports Nutr. 2012;9(1):P5.
13. Panton LB, Rathmacher JA, Baier S, Nissen S. Nutritional supplementation of the leucine metabolite β-hydroxy-β-methylbutyrate (hmb) during resistance training. Nutrition. 2000;16(9):734-9.
14. Asadi A, Arazi H, Suzuki K. Effects of β-hydroxy-β-methylbutyrate-free acid supplementation on strength, power and hormonal adaptations following resistance training. Nutrients. 2017;9(12):1316.
15. Durkalec-Michalski K, Jeszka J, Podgórski T. The effect of a 12-week beta-hydroxy-beta-methylbutyrate (HMB) supplementation on highly-trained combat sports athletes: A randomised, double-blind, placebo-controlled crossover study. Nutrients. 2017;9(7):753.
16. Durkalec-Michalski K, Jeszka J. The effect of β-hydroxy-β-methylbutyrate on aerobic capacity and body composition in trained athletes. J Strength Cond Res. 2016;30(9):2617-26.
17. Durkalec-Michalski K, Jeszka J. The efficacy of a β-hydroxy-β-methylbutyrate supplementation on physical capacity, body composition and biochemical markers in elite rowers: A randomised, double-blind, placebo-controlled crossover study. J Int Soc Sports Nutr. 2015;12(1):31.
18. Portal S, Zadik Z, Rabinowitz J, Pilz-Burstein R, Adler-Portal D, Meckel Y, et al. The effect of HMB supplementation on body composition, fitness, hormonal and inflammatory mediators in elite adolescent volleyball players: A prospective randomized, double-blind, placebo-controlled study. Eur J Appl Physiol. 2011;111(9):2261-9.
19. Correia ALM, de Lima FD, Bottaro M, Vieira A, da Fonseca AC, Lima RM. Pre-exercise β-hydroxy-β-methylbutyrate free-acid supplementation improves work capacity recovery: A randomized, double-blinded, placebo-controlled study. Appl Physiol Nutr Metab. 2018(999):1-6.
20. Gonzalez AM, Stout JR, Jajtner AR, Townsend JR, Wells AJ, Beyer KS, et al. Effects of β-hydroxy-β-methylbutyrate free acid and cold water immersion on post-exercise markers of muscle damage. Amino Acids. 2014;46(6):1501-11.
21. Wilson JM, Lowery RP, Joy JM, Walters JA, Baier SM, Fuller JC, et al. β-Hydroxy-β-methylbutyrate free acid reduces markers of exercise-induced muscle damage and improves recovery in resistance-trained men. Br J Nutr. 2013;110(3):538-44.
22. Knitter A, Panton L, Rathmacher J, Petersen A, Sharp R. Effects of β-hydroxy-β-methylbutyrate on muscle damage after a prolonged run. J Appl Physiol. 2000;89(4):1340-4.
23. Engelen MP, Deutz NE. Is β-hydroxy β-methylbutyrate an effective anabolic agent to improve outcome in older diseased populations? Curr Opin Clin Nutr Metab Care. 2018;21(3):207-13.
24. Silva VR, Belozo FL, Micheletti TO, Conrado M, Stout JR, Pimentel GD, et al. β-Hydroxy-β-methylbutyrate free acid supplementation may improve recovery and muscle adaptations after resistance training: A systematic review. Nutr Res. 2017;45:1-9.

25. Albert FJ, Morente-Sánchez J, Ortega FB, Castillo MJ, Gutiérrez Á. Usefulness of β-hydroxy-β-methylbutyrate (HMB) supplementation in different sports: An update and practical implications. Nutr Hosp. 2015;32(1):20-33.
26. Wilson JM, Fitschen PJ, Campbell B, Wilson GJ, Zanchi N, Taylor L, et al. International Society of Sports Nutrition Position Stand: Beta-hydroxy-beta-methylbutyrate (HMB). J Int Soc Sports Nutr. 2013;10(1):6.
27. Zanchi NE, Gerlinger-Romero F, Guimaraes-Ferreira L, de Siqueira Filho MA, Felitti V, Lira FS, et al. HMB supplementation: Clinical and athletic performance-related effects and mechanisms of action. Amino Acids. 2011;40(4):1015-25.
28. Garlick PJ. The role of leucine in the regulation of protein metabolism. J Nutr. 2005;135(6): 1553S-6S.
29. Pinheiro CHJ, Guimaraes-Ferreira L, Gerlinger-Romero F, Curi R. An overview on beta-hydroxy-beta-methylbutyrate (HMB) supplementation in skeletal muscle function and sports performance. Nutrition and Enhanced Sports Performance: Elsevier. 2014;455-63.
30. Sabourin PJ, Bieber LL. Formation of beta-hydroxyisovalerate by an alpha-ketoisocaproate oxygenase in human liver. Metabolism. 1983;32(2):160-4.
31. Van Koevering M, Nissen S. Oxidation of leucine and alpha-ketoisocaproate to beta-hydroxy-beta-methylbutyrate *in vivo*. Am J Physiol Endocrinol Metab. 1992;262(1):E27-E31.
32. Nissen SL, Abumrad NN. Nutritional role of the leucine metabolite β-hydroxy β-methylbutyrate (HMB). J Nutr Biochem. 1997;8(6):300-11.
33. Fuller JC, Sharp RL, Angus HF, Baier SM, Rathmacher JA. Free acid gel form of β-hydroxy-β-methylbutyrate (HMB) improves HMB clearance from plasma in human subjects compared with the calcium HMB salt. Br J Nutr. 2011;105(3):367-72.
34. Vukovich MD, Slater G, Macchi MB, Turner MJ, Fallon K, Boston T, et al. β-hydroxy-β-methylbutyrate (HMB) kinetics and the influence of glucose ingestion in humans. J Nutr Biochem. 2001;12(11):631-9.
35. Fuller JC, Sharp RL, Angus HF, Khoo PY, Rathmacher JA. Comparison of availability and plasma clearance rates of β-hydroxy-β-methylbutyrate delivery in the free acid and calcium salt forms. Br J Nutr. 2015;114(9):1403-9.
36. Wilkinson D, Hossain T, Hill D, Phillips B, Crossland H, Williams J, et al. Effects of leucine and its metabolite β-hydroxy-β-methylbutyrate on human skeletal muscle protein metabolism. J Physiol. 2013;591(11):2911-23.
37. Shreeram S, Johns PW, Subramaniam S, Ramesh S, Vaidyanathan V, Puthan JK, et al. The relative bioavailability of the calcium salt of β-hydroxy-β-methylbutyrate is greater than that of the free fatty acid form in rats–3. J Nutr. 2014;144(10):1549-55.
38. Kornasio R, Riederer I, Butler-Browne G, Mouly V, Uni Z, Halevy O. β-hydroxy-β-methylbutyrate (HMB) stimulates myogenic cell proliferation, differentiation and survival via the MAPK/ERK and PI3K/Akt pathways. Biochim Biophys Acta Mol Cell Res. 2009;1793(5):755-63.
39. Gerlinger-Romero F, Guimarães-Ferreira L, Giannocco G, Nunes M. Chronic supplementation of beta-hydroxy-beta methylbutyrate (HMβ) increases the activity of the GH/IGF-I axis and induces hyperinsulinemia in rats. Growth Horm IGF Res. 2011;21(2):57-62.
40. Smith HJ, Mukerji P, Tisdale MJ. Attenuation of proteasome-induced proteolysis in skeletal muscle by β-hydroxy-β-methylbutyrate in cancer-induced muscle loss. Cancer Res. 2005;65(1): 277-83.
41. Kovarik M, Muthny T, Sispera L, Holecek M. Effects of β-hydroxy-β-methylbutyrate treatment in different types of skeletal muscle of intact and septic rats. J Physiol Biochem. 2010;66(4):311-9.
42. Eley HL, Russell ST, Tisdale MJ. Attenuation of depression of muscle protein synthesis induced by lipopolysaccharide, tumor necrosis factor and angiotensin II by β-hydroxy-β-methylbutyrate. Am J Physiol Endocrinol Metab. 2008;295(6):E1409-E16.
43. Hao Y, Jackson JR, Wang Y, Edens N, Pereira SL, Alway SE. β-Hydroxy-β-methylbutyrate reduces myonuclear apoptosis during recovery from hind limb suspension-induced muscle fiber atrophy in aged rats. Am J Physiol Regul Integr Comp Physiol. 2011;301(3):R701-R15.
44. Holeček M. Beta-hydroxy-beta-methylbutyrate supplementation and skeletal muscle in healthy and muscle-wasting conditions. J Cachexia Sarcopenia Muscle. 2017;8(4):529-41.
45. Nissen S, Sharp R, Ray M, Rathmacher J, Rice D, Fuller Jr J, et al. Effect of leucine metabolite β-hydroxy-β-methylbutyrate on muscle metabolism during resistance-exercise training. J Appl Physiol. 1996;81(5):2095-104.

46. Vallejo J, Spence M, Cheng A-L, Brotto L, Edens NK, Garvey SM, et al. Cellular and physiological effects of dietary supplementation with β-hydroxy-β-methylbutyrate (HMB) and β-alanine in late middle-aged mice. PLoS One. 2016;11(3):e0150066.
47. He X, Duan Y, Yao K, Li F, Hou Y, Wu G, et al. β-Hydroxy-β-methylbutyrate, mitochondrial biogenesis, and skeletal muscle health. Amino Acids. 2016;48(3):653-64.
48. da Justa Pinheiro CH, Gerlinger-Romero F, Guimaraes-Ferreira L, de Souza-Jr AL, Vitzel KF, Nachbar RT, et al. Metabolic and functional effects of beta-hydroxy-beta-methylbutyrate (HMB) supplementation in skeletal muscle. Eur J Appl Physiol. 2012;112(7):2531-7.
49. Peterson A, Qureshi M, Ferket P, Fuller Jr J. *In vitro* exposure with β-hydroxy-β-methylbutyrate enhances chicken macrophage growth and function. Vet Immunol Immunopathol. 1999;67(1): 67-78.
50. Clark RH, Feleke G, Din M, Yasmin T, Singh G, Khan FA, et al. Nutritional treatment for acquired immunodeficiency virus-associated wasting using β-hydroxy β-methylbutyrate, glutamine and arginine: A randomized, double-blind, placebo-controlled study. J Parenter Enteral Nutr. 2000;24(3):133-9.
51. Hsieh L, Chien S, Huang M, Tseng H, Chang C. Anti-inflammatory and anticatabolic effects of short-term beta-hydroxy-beta-methylbutyrate supplementation on chronic obstructive pulmonary disease patients in intensive care unit. Asia Pac J Clin Nutr. 2006;15(4):544-50.
52. Roberts L, Suzuki K. Exercise and inflammation. Antioxidants. 2019;8(6):155-8.
53. Suzuki K. Cytokine response to exercise and its modulation. Antioxidants. 2018;7(1):17.
54. Ma S, Suzuki K. Toll-like receptor 4: Target of lipotoxicity and exercise-Induced anti-inflammatory effect? Ann Nutr Food Sci. 2018;2(4):1-3.
55. Clowes Jr GH, George BC, Villee Jr CA, Saravis CA. Muscle proteolysis induced by a circulating peptide in patients with sepsis or trauma. N Engl J Med. 1983;308(10):545-52.
56. Townsend JR, Fragala MS, Jajtner AR, Gonzalez AM, Wells AJ, Mangine GT, et al. β-Hydroxy-β-methylbutyrate (HMB)-free acid attenuates circulating TNF-α and TNFR1 expression postresistance exercise. J Appl Physiol. 2013;115(8):1173-82.
57. Gonzalez AM, Fragala MS, Jajtner AR, Townsend JR, Wells AJ, Beyer KS, et al. Effects of β-hydroxy-β-methylbutyrate free acid and cold water immersion on expression of CR3 and MIP-1β following resistance exercise. Am J Physiol Regul Integr Comp Physiol. 2014;306(7):R483-R9.
58. Hoffman JR, Gepner Y, Stout JR, Hoffman MW, Ben-Dov D, Funk S, et al. β-Hydroxy-β-methylbutyrate attenuates cytokine response during sustained military training. Nutr Res. 2016;36(6):553-63.
59. Teixeira FJ, Matias CN, Monteiro CP, Valamatos MJ, Reis JF, Morton RW, et al. Leucine metabolites do not attenuate training-induced inflammation in young resistance trained men. J Sports Sci. 2019;37(17):2037-44.
60. Vulcan PR. Role of β-Hydroxy-β-methylbutyrate (HMB) on inflammation after eccentric exercise. Master's Thesis. Iowa State University, Ames, Iowa, USA; 2012.
61. Arazi H, Asadi A, Suzuki K. The effects of beta-hydroxy-beta-methylbutyrate-free acid supplementation and resistance training on oxidative stress markers: A randomized, double-blind, placebo-controlled study. Antioxidants. 2018;7(6):76.
62. Arazi H, Hosseini Z, Asadi A, Ramirez-Campillo R, Suzuki K. β-hydroxy-β-methylbutyrate-free acid attenuates oxidative stress induced by a single bout of plyometric exercise. Front Physiol. 2019;10:776.
63. Rudney H. The biosynthesis of β-hydroxy-β-methylglutaric acid. J Biol Chem. 1957;227(1):363-77.
64. Nissen S, Sharp R, Panton L, Vukovich M, Trappe S, Fuller Jr J. β-hydroxy-β-methylbutyrate (HMB) supplementation in humans is safe and may decrease cardiovascular risk factors. J Nutr. 2000;130(8):1937-45.
65. Hsieh L-C, Chow C-J, Chang W-C, Liu T-H, Chang C-K. Effect of β-hydroxy-β-methylbutyrate on protein metabolism in bed-ridden elderly receiving tube feeding. Asia Pac J Clin Nutr. 2010;19(2):200-8.
66. Holecek M, Muthny T, Kovarik M, Sispera L. Effect of beta-hydroxy-beta-methylbutyrate (HMB) on protein metabolism in whole body and in selected tissues. Food Chem Toxicol. 2009;47(1):255-9.

67. Sharawy MH, El-Awady MS, Megahed N, Gameil NM. The ergogenic supplement β-hydroxy-β-methylbutyrate (HMB) attenuates insulin resistance through suppressing GLUT-2 in rat liver. Can J Physiol Pharmacol. 2015;94(5):488-97.
68. Ravanbakhsh N, Torabi N, Foadoddini M. Preventive effects of beta-hydroxy-beta-methyl butyrate on cardiac arrhythmias in rats (Abstract). Ofogh-E-Danesh. 2016;22(3):209-14. [In persian].
69. Aghaei F, Shadmehri S, Soleymani A. Effects of resistance training and HMB supplementation on responses of hs-CRP and lipid profiles to strenuous activity in adult male rats (Abstract). Iran J Nutr Sci Food Tech. 2019;14(3):31-40. [In persian].
70. Arazi H, Rohani H, Ghiasi A, Davaran M. The effect of HMB supplementation on cardiovascular risk factors after four weeks of resistance training in amateur athletes. Int Cardiovasc Res J. 2015;9(2):89-93.
71. Baptista DB, de Santana LFD, Veras ASC, de Menezes VA, dos Santos NJ, Papoti M, et al. Effect of concurrent training and supplementation with β-Hydroxy-β-Methylbutyirate (HMB) on the prostate: Alterations in the androgen receptor and inflammation. Int J Morphol. 2018;36(1):74-9.
72. Suzuki K. Chronic inflammation as an immunological abnormality and effectiveness of exercise. Biomolecules. 2019;9(6):223.
73. Suzuki K, Tominaga T, Ruhee RT, Ma S. Characterization and modulation of systemic inflammatory response to exhaustive exercise in relation to oxidative stress. Antioxidants. 2020;9(5):401.

Biography of author(s)



Hamid Arazi
Department of Exercise Physiology, Faculty of Sport Sciences, University of Guilan, Rasht 4199843653, Iran.

He is a professor at the University of Guilan (Iran). He is specialized in resistance training, athlete conditioning, nutritional supplements and health. He received a Ph.D degree in exercise physiology, from University of Guilan (Faculty of Sport Sciences) in Iran. His research works involve athlete conditioning, muscular adaptations, health promotion, nutritional supplements and ergogenic aids and teaching activities are in the areas of theory (such as exercise biochemistry, exercise physiology, conditioning athletes, drugs abuse in athletes, exercise and disease management and prevention) and practical classes (including physical fitness and exercise physiology laboratory). He has published as author or co-author numerous papers in peer-reviewed journals, has conducted many research projects and has held a lot of workshops in scope of exercise physiology (athlete performance enhancement, physical fitness, ergogenic supplements and health). Moreover, He has been plenary speaker in several scientific conferences at the national or international level. Currently he is serving as a reviewer or review editor for reputable and high quality scientific journals. Also, he is an editorial board member of prestigious international journals and received the awards as eminent researcher.



Behzad Taati
Department of Exercise Physiology, Faculty of Sport Sciences, University of Guilan, Rasht 4199843653, Iran.

He is a PhD candidate in exercise physiology at the University of Guilan, Iran. He earned a master's degree in exercise physiology from this University in 2014. He has published scientific articles in journals such as *antioxidants*, *Frontiers in Nutrition*, *Journal of Health, Population and Nutrition*, *Journal of Women & Aging*, and *Comparative Exercise Physiology*. His current research interests include sports nutrition, exercise training and health, and sports performance. He is currently involved in PhD research on taekwondo athletes and their physical fitness testing.



Katsuhiko Suzuki
Faculty of Sport Sciences, Waseda University, Tokorozawa 359-1192, Japan.

He is a Professor, a Cooperative Major in Advanced Health Sciences, Tokyo University of Agriculture and Technology and Waseda University (2010-2013); Adjunct Professor, Institute of Biomedical Innovation, School of Biomedical Sciences, Faculty of Health, Queensland University of Technology, Australia (2013-2014); President, International Society of Exercise and Immunology (ISEI) (2015-2017) and the board member on and after; Guest Editor, "Exercise and Inflammation" of Antioxidants (2017-2018) and "Anti-inflammatory and Antioxidant Effects of Dietary Supplementation and Lifestyle factors" of Antioxidants (2019-2020) and Associate Editor of Frontiers in Physiology (2019-Present). Currently, his research themes are: Risk assessment of acute and chronic exercise using biochemical and immunological variables, analysis and assessment of

immunity and inflammation centered on phagocytes and cytokines, role of free radicals and antioxidants in health and diseases, exercise prescription for lifestyle-related diseases, and assessment of anti-oxidative and anti-inflammatory food components. His education history is as follow: BSc (Human Sciences), Waseda University, Japan, 1991; MSc (Human Sciences), Waseda University, Japan, 1993; MD (Medical Doctor), Hirosaki University School of Medicine, Japan, 1999; and PhD (Medicine), Hirosaki University School of Medicine, Japan, 2002. He has worked as General Physician, Hospital, International Medical Center of Japan (1999–2001), Instructor, Hirosaki University School of Medicine, Japan (2002–2003), Assistant Professor, Faculty of Human Sciences, Waseda University, Japan (2003–2008), Associate Professor, Faculty of Sport Sciences, Waseda University, Japan (2008–2013), and Professor, Faculty of Sport Sciences, Waseda University, Japan (2013 –Now).

© Copyright (2021): Author(s). The licensee is the publisher (Book Publisher International).

Chapter 12
Print ISBN: 978-93-90516-93-3, eBook ISBN: 978-93-90516-94-0

Isolation of Dermatophytes from Superficial Fungal Infections at Badaun, Uttar Pradesh

Pankaj Saxena[1*]

DOI: 10.9734/bpi/rrab/v5/8246D

ABSTRACT

The most common causative agents of superficial infections are dermatophytic fungi and other non dermatophytic fungi. The present work focuses on isolation and identification of dermatophytes and non dermatophytes from superficial mycoses. One hundred Fifty (150)clinical specimens were subjected to firstly potassium hydroxide (KOH) examination and then culture isolation on SDA media. Identification of dermatophytes on the basis of macroscopic and microscopic characteristics. Among 150 suspected patients samples of dermatophytosis, 130 (86%) samples were found positive by KOH mount and 90 (60%) were culture positive cases. Trichophyton mentagrophyte was the most frequent isolate from clinical types of dermatophytosis followed by *Trichophyton rubrum*, *Microsporum audouinii*, *Microsporum canis* & *Microsporum gypseum*. Dermatophytes were well grow at high temperature, moisture and humidity. Personal hygienc and living conditions are also important factors which are helpful to cause dermatophytosis. Trichophyton species was found to be the most common most etiological agent of dermatophytosis in the current study.

Keywords: Dermatophytes; culture positive; fungal infection; dermatophytosis.

1. INTRODUCTION

Superficial Mycoses are the strictly surface infections involving skin, hair, nails and mucosa. It is caused by dermatophytes that infect only keratin rich tissues without involving the living tissue and they are incapable of penetrating subcutaneous tissues. Ringworm or Dermatophytosis is found world-wide with most prevalence in hot climatic countries [1]. These are the group of fungi that are keratin loving like to eat keratin present in outer epidermal layers of skin, hair and nails in human beings. They are very pathogenic fungi that obtain their food from keratin rich tissues from living cells. They are parasitic in nature [2]. Such type of infections spreads by contact, it may be direct or indirect, from infected humans and animals (direct) or through indirect contact such as inanimate objects and fomites [3]. Dermatophytes are classified into three genera, i.e Trichophyton, Microsporum and Epidermophyton. These genera belong to Class Hphomycetes and Division Deuteromycota. Otherfactors responsible for dermatophytosis is high sweating during summer, contact with animals, use of unhygienic towels, clothes and wearing of tight undergarments. Due to this major factor dermatophytosis was a very common superficial fungal infection of human beings in India [4]. Sometimes infection may also be caused by Candida species and non-dermatophytes. Non dermatophytes belong to the genera Fusarium, Scopulariopsis and Aspergillus [5]. Proper diagnosis of disease was required proper laboratory diagnosis. Culture examination was the crucial step after KOH mount to identify the accurate causative agent. In some cases, on the basis of clinical symptoms, specific identification of invasive mould will be carried out. Some nail and skin infection, also reported which are caused by non-dermatophytes molds which are often resistant to anti dermatophytic drugs [6]. Candidiasis is the most common fungal infection mostly caused by yeast like fungus *Candida albicans* in 90% cases. Such type of infections are called Candidiasis. *C. albicans* was also normal microbial flora of healthy human body but when human body immune system is weak

[1]*Department of Agriculture & Veterinary Science, Jayoti Vidyapeeth Women's University, Jaipur, India.*
**Corresponding author: E-mail: kamalrocks3@gmail.com;*

and suppressed due to old ages and use of immunosuppressive drugs, *Candida ablicans* was converted into pathogenic form and cause disease known as Candidiasis [7,8].

2. MATERIALS AND METHODS

One hundred Fifty (150) clinical samples were collected from Maheshwari Diagnostic Centre and Hospital, Badaun in a black polyethene bags. Clinical specimens collected were skin scrapings, hair clippings and nail. All collected clinical specimens were processed for diagnosis [6,9]. Direct Microscopic Examination (KOH mount) and culture on SDA media and other selective media were used for lab diagnosis of dermatophytosis disease [6,10,11,12]. Identification of dermatophytes and other molds was carried by the following methods:-

1. Direct Microscopic by KOH mount [9].
2. Culture: Triplicates sets of media were prepared for culture on SDA Media irrespective of KOH examination.

    a) Sabouraud's Dextrose Agar (SDA)
    b) SDA media supplemented with antibiotic chloramphenicol to avoid contamination of other saprophytic fungi and bacteria;
    c) Selective media for isolation of Dermatophytes used was Dermatophyte Test Medium (DTM)

3. After culture other microscopic test were: Lacto phenol cotton blue mount (LCBM) and Slide culture technique

3. RESULTS AND DISCUSSION

Dermatophytosis was found to be the common most superficial fungal infection in 90 culture positive cases (60%) as shown in Tables 1 & 2. This study was similar with other researchers Karmakar et al. [13], Bindu & Pavithran, [14], Singh & Beena, [4] who also reported dermatophytosis was the common superficial fungal infections in different areas of India. Different species of dermatophytes were isolated in the present study (Table 3). In this work, predominant etiological agent of dermatophytosis was Trichophyton species than the Microsporum and Epidermophyton. In the present study, among all clinical types of dermatophytosis, *Tinea corporis* was reported the most common clinical type. These findings match with the other researchers who also found *Tinea corporis* was the predominant clinical type of dermatophytosis among all clinical types [15,16,14]. The current study proves that high isolation rate (77%) was found in *Tinea corporis* followed by tineacruris (50%). In the present study, Trichophytonment agrophytes was isolated from all clinical types of dermatophytos is except *Tine amanum* and *Tinea barbae* and reported to be the predominant etiological agent of dermatophytosis as shown in Table 3. *T. mentagrophyte* was isolated from 45 (50%) patients out of 90 culture positive cases, This is in contrast to other studies by Siddappa et al. [17] and Singh & Beena [4] where *T. rubrum* was most common dermatophyte isolated from clinical specimens. Current study coincides with the Agarwal et al. [18] found in his studies, *T. mentagrophytes* was reported common dermatophyte among all isolates. Major prevalence of *T. mentagrophytes* in this area (Badaun) due to more labour class population working in hot and humid climatic conditions. The present study also coincides with the Nasimuddin et al. [19] who also reported in his study, the majority of the isolates were *Trichophyton mentagrophytes* (38.75%) followed by *Trichophyton rubrum* (11.6%). Maximum number of isolates of *T. mentagrophytes* were obtained from *Tinea corporis* clinical type of dermatophytosis. Twenty Eight (28) isolates were obtained from *Tinea corporis* followed by *Tinea cruris*, *Tinea capitis*, *Tinea pedis* and *Tinea faciei*. No Isolate of *T. mentagrophyte* was found to be from *Tinea manuum* & *Tinea barbae* (Table 3). In new era, Increasing prevalence of *T. mentagrophytes* was reported by many workers. The present study matches with the findings of earlier workers who reported *Trichophytonment agrophytes* was dominant species isolated in his study (48.3%) [20,21,22]. In *Tinea capitis*, the predominant isolate was *T. tonsurans* with 3 cases followed by *T. rubrum*, *T. mentagrophytes*, *M. gypseum* and *M. audouinii*. This differs from the study by Kalla et al. [23] where *T. violaceum* was the predominant isolate and matches with the Belurkar et

al. [24] found *T. tonsurans* as the predominant isolate (57.1%) from *Tinea capitis*. Such differences could be due to geographical variations of various dermatophytes and specific affinity of some species of dermatophytes for specific anatomical area. The second etiological agent was reported *T. rubrum* from *Tinea corporis*, *Tinea cruris*, *Tinea capitis* and *Tinea pedis* clinical types except *Tinea manuum*, *Tinea faciei* and *Tinea barbae*. The third etiological agents was *Microsporum audouinii* isolated from all clinical types except *Tinea faciei* & *Tinea barbae* from 7 cases (7.7%) out of 90 culture positive cases.

Microsporum canis was reported fourth causative agent of dermatophytosis in the present study isolated from skin scrapings. *M. gypseum* was reported fifth causative agent of dermatophytosis from hair and skin. One (1) isolate of *T. tonsurans* and *Chrysosporium tropicumeach* were isolated from *Tinea corporis* patients. *Microsporum audouinii* was found to be the etiological agent of *Tinea manuum* in the current study. Highest Culture positive cases were reported in *Tinea corporis* &low culture positive cases with *Tinea manuum*, *Tinea barbae* and *Tinea faciei*. Present study coincides with Jain et al. [25] found in her study, highest prevalence of culture positive cases in *Tinea corporis* clinical type.

Table 1. Culture and KOH positive results of Dermatophytosis

Mycosis	Total samples	KOH +ve	% KOH +ve	Culture +ve	% Culture +ve
Dermatophytosis	150	130	86%	90	60%

Table 2. Culture positive cases of various clinical types of Dermatophytosis

Clinical types	Total no. of cases	No. of KOH +ve cases	% KOH+ve cases	No. of culture +ve cases	% of culture +ve cases
Tinea corporis	75	73	97%	58	77%
Tinea cruris	30	27	90%	15	50%
Tinea capitis	20	15	75%	9	45%
Tinea pedis	15	10	66%	6	40%
Tinea manum	5	3	60%	1	33%
Tinea faciei	3	2	66%	1	33%
Tinea barbae	2	1	50%	0	0%
Total	**150**	**130**	**86%**	**90**	**60%**

Table 3. Incidence of dermatophytes from different clinical types of dermatophytosis

Clinical type	*T. mentagrophyte*		*T. rubrum*		*M. audouini*		*M. canis*		*M. gypseum*		*T. tonsurans*		*C. tropicum*	
Tinea corporis	28	31%	15	16.6%	3	3.3%	2	2.2%	1	1.1%	8	8.8%	1	1.1%
Tinea cruris	12	13.3%	1	1.1%	1	1.1%	1	1.1%	-	-	-	-	-	-
Tinea capitis	2	2.2%	2	2.2%	1	1.1%	-	-	1	1.1%	3	3.3%	-	-
Tinea pedis	2	2.2%	2	2.2%	1	1.1%	1	1.1%	-	-	-	-	-	-
Tinea manuum	-	-	-	-	1	1.1%	-	-	-	-	-	-	-	-
Tinea faciei	1	1.1%	-	-	-		-	-	-	-	-	-	-	-
Tinea barbae	-	-	-	-	-		-	-	-		-	-	-	-
Total	45	50%	20	22.2%	7	7.7%	4	4.4%	2	2.2%	11	12.2%	1	1.1%

4. CONCLUSION

In the present study, fungi were demonstrated by KOH and culture method in 90 cases (60%) out of 150 cases. Highest isolation rate by culture was 60%. The most common isolate obtained was *T. mentagrophytes* (50%). Second common dominant species after *T. mentagrophyte* was *T. rubrum* (22.2%). Most of the studies have reported major dermatophyte was *T. rubrum* from dermatophytosis but in the current findings reported *T. mentagrophyte* as the predominant dermatophyte. The present

study reveals the variation in prevalence of various dermatophyte species due to geographical locations and climatic conditions in Badaun.

COMPETING INTERESTS

Author has declared that no competing interests exist.

REFERENCES

1. Ranganathan S, Menon T, Sentamil GS. Effect of socioeconomic status on the prevalence of dermatophytosis in Madras. Indian J DermatolVenereolLeprol. 1995;61:16-8.
2. Weitzman, I. and Summerbell, R. The dermatophytes. Clinical Microbiol Review. 1995;8:240-259.
3. Nweze EI. Dermatophytosis in Western Africa: a review. Pak J Biol Sci. 2010;13:649-56
4. Singh S, Beena PM. Comparative study of different techniques and culture media for the isolation of dermatophytes. Indian J Med Microbiol, 2003;21:21-24.
5. Naveed AM, Naeem R, Nasiruddin. Non- dermatophyte moulds and yeasts as causative agents in Onychomycosis. Journal of Pakistan Association of Der- matologists. 2009;19(2):74-78.
6. Padhye AA, Weitzman I: The Dermatophytes. In: Topley & Wilson's Microbiology and Microbial Infection (Medical Mycology ;vol. IV) L Ajello, RJ Hay, L Collier, A Balows, M Sussman (Eds.); 9th Edn.; Arnold publication, Great Britain, 1998;215-225.
7. Vázquez-González D, Perusquía-Ortiz AM, Hundeiker M, Bonifaz A. Opportunistic yeast infections: candidiasis, cryptococcosis, trichosporonosis and geotrichosis. J. Ger. Soc. Dermatology. 2013;11:381–94.
8. Greenberg MS, Glick M. Burket's Oral Medicine: Diagnosis and Treatment. 10 Edition. USA: BC Decker Inc.; 2003; p. 658.
9. Chander J. Dermatophytoses. In: Textbook of Medical Mycology. 3rd Edn.; Mehta publication, New Delhi, 2009;134-141.
10. Hay RJ, Ashbee HR: Mycology (Chapter 36). In: Rook's Text book of Dermatology (vol. II). B Tony, B Stephen , C Neil, G Christopher, (Eds.); 8th Edn.; Willey Blackwell, Oxford. 2010;18-20.
11. Emmons CW, Chapman HB, John PU. Dermatophytoses. In: Medical Mycology. 2nd, Edn.; Henry Klipton Publishers, Great Britain. 1970;109-120.
12. Rippons JW. Pathogenic fungi and pathogenic actinomycetes. In: Medical Mycology. KJ Kwon Chung & JE Bennett: (Eds.); 3rd Edn.; WB Saunders Company, Philadelphia. 1988;230-239.
13. Karmakar S, Kalla G, Joshi KR, Karmakar S. Dermatophytosis in a desert district of Western Rajasthan. Indian Journal of Dermatology, Venereology & Leprology. 1995;61(5):280-283.
14. Bindu V, Pavithran K. Clinico-mycological study of dermatophytosis in Calicut. Indian J Dermatol Venereol Leprol. 2002;68:259-61.
15. Kannan P, Janaki C, Selvi GS. Prevalence of dermatophytes and other fungal agents isolated from clinical samples. Ind J Med Microbiol .2006;24(3):212-215.
16. Rajesh R, Subramainam K, Padmavathy BK, Vasanthi S. Prevalence and species profile of Dermatophytosis among HIV positive patients in ruran referral centre. Indian J Transm disease 2006;27(2):70-74.
17. Siddappa K, Mahipal OA. Dermatophytoses in Davangere. Indian J Dermatol Venereol Leprol. 1982;48:254-9.
18. Agarwal US, Saran J, Agarwal P. Clinico-mycological study of dermatophytes in a tertiary care centre in northwest India. Indian J Dermatol Venereol Leprol. 2014;80:194.
19. Nasimuddin Sowmya, Appalaraju B, Surendran P, Srinivas CR. Isolation, identification and comparatative analysis of SDA and DTM for dermatophytes from clinical samples in a tertiary care hospital, IOSR Journal of Dental and Medical Sciences. 2014;13 (11):68-73.
20. Kumar K, Kindo AJ, Kalyani J, Anandan S. Clinico–Mycological profile of dermatophytic skin infections in a Tertiary Care Center–A cross sectional study. Sri Ramachandra Journal of Medicine. 2007;1(2);12-5.
21. Venkatesan G, Ranjit Singh AJA, Murugesan AG, Janaki C, Gokul Shankar S. *Trichophyton rubrum*– The predominant etiological agent in human dermatophytoses in Chennai, India. Afr J Microbiol Res. 2007;1: 9-12.

22. Balakumar Srinivasan. Epidemiology of dermatophytosis in and around Tiruchirapalli, Tamilnadu, India. Asian Pac J Trop Dis. 2012;2(4):286-9
23. Kalla G, Begra B, Solanki A, Goyal A, Batra A. Clinicomycological study of *Tinea capitis* in desert district of Rajasthan. Indian J Dermatol Venereol Leprol. 1995;61:342-5.
24. Belurkar DD, Bharmal RN, Kartikeyan S, Vadhavkar RS. A mycological study of Dermatophytoses in Thane. Bombay Hospital Journal. 2004;52:311-312.
25. Jain N, Sharma M, Saxena VN. Clinicomycological profile of dermatophytosis in Jaipur Rajasthan. Indian J Dermatol Leprol Venereol. 2008;74(3):274-275.

Biography of author(s)



Pankaj Saxena
Department of Agriculture & Veterinary Science, Jayoti Vidyapeeth Women's University, Jaipur, India.

Research and Academic Experience: 5-6 years

Research Area: Medical Microbiology

Number of Published papers: 5

© Copyright (2021): Author(s). The licensee is the publisher (Book Publisher International).

London Tarakeswar

Registered offices
India: Guest House Road, Street no - 1/6, Hooghly, West Bengal, PIN-712410, India, Corp. Firm Registration Number: L77527, Tele: +91 8617752708, Email: director@bookpi.org, (Headquarters)
UK: Third Floor, 207 Regent Street, London, W1B 3HH, UK
Fax: +44 20-3031-1429 Email: director@bookpi.org, (Branch office)